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

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EDITOR-IN-CHIEF'S PREFACE

EDITOR-IN-CHIEF'S PREFACE TO ENGLISH VERSION OF ISSUE 1, 2023

Sergey I. Kolesnikov

Member of Russian Academy of Sciences

Dear readers!

Having studied the articles presented in this issue, I was not able to single out one leading article, since most of the published works are works of approximately the same, high level. Therefore, I will focus on those articles that may be of interest not only to narrow specialists, but also to a wider readership.

I will start with articles related to infectious pathology, which has not left the front pages of both scientific journals and popular publications. This applies to both COVID-19 and its consequences, as well as the newly threatening A/H1N1 influenza.

First of all, this is an article by D.D. Bryukhova et al. (Irkutsk) on the dynamics of humoral immunity in children during the spread of COVID-19. They proved the high intensity of the hidden epidemic process and the formation of relatively stable immunity, which, in my opinion, requires a separate discussion in terms of the proposed vaccination of the child population. In the work of T.I. Petelina et al. (Tyumen) it has been shown that the nature of the development of both psychological and cardiovascular complications after COVID-19 is determined by a prolonged vascular response, a violation of the rheological and metabolic properties of blood.

Naturally, the spread of infections requires the use of methods for assessing their possible dynamics, the formation of immunity and the correction of emerging disorders. Article by A.V. Malyarchikov and K.G. Shapovalov (Chita) is devoted to the increased risk of developing organ failure in severe pneumonia with *TLR4* Asp299Gly gene polymorphism in influenza A/H1N1. And in the work of V.Yu. Malygina et al. (Simferopol) revealed that the introduction of tauroside Sx1 saponin positively affects the functional activity of the spleen when mice are infected with the H1N1 influenza virus.

The review of A.M. Ziganshin et al. (Ufa, Chita) proves the prospects of using CRISPR-Cas9 technology in the prevention and treatment of certain viral diseases. Article by E.Yu. Zyablitskaya with co-authors-scientists and medical practitioners from Simferopol and Moscow showed the effectiveness of using the TigraTest® SARS-CoV-2 ELISPOT method to detect latent forms of coronavirus infection.

The problem of tuberculosis, including its comorbidity with HIV infection, does not leave the scientific agenda of our country, which is the subject of the article by E.A. Borodulina et al. (Samara). There is also a problem with the diagnosis of pseudotuberculosis and its outbreaks, as evidenced by the article by T.Yu. Zagoskina et al. (Irkutsk) on the use of dot-immunoassay for express identification of *Y. pseudotuberculosis* cultures.

It seems to me that the attention of readers will be attracted by the article by N.B. Semenova et al. (Krasnoyarsk, Novosibirsk) on the prevalence of psychosomatic comorbidity of eating disorders and especially subthreshold eating disorders in schoolgirls aged 11–17 years. A feature of clinical manifestations is the frequent absence of underweight and comorbidity with pain. I hope that you will also be interested in the article by E.A. Gorobets and O.R. Esin (Kazan) regarding the relationship between the level of alexithymia in adolescents and various somatic diseases, which should be taken into account when assessing the level of alexithymia and central sensitization in adolescents with headaches.

Several articles are devoted to the development and use of methods of non-drug intervention in various diseases and their modeling. These are the works of N.S. Tribrat et al. (Simferopol, Kazan) on the effect of a glucose-electrolyte drink with plant antioxidants on the performance of the arteries of the vertebrobasilar basin after a step-increasing load. Article by A.E. Shoboev et al. (Bokhan, Ir-

kutsk) proves that the method of reflexology in comparison with physiotherapy is more effective for restoring the function of swallowing in the post-stroke period.

Several articles are devoted to surgical problems and their experimental modeling. The work of M.M. Bikbov et al. (Ufa) shows the effectiveness of dosed muscle-conjunctival resection of the cartilage of the upper eyelid in patients with partial ptosis of the upper eyelid. I would also single out the work of M.S. Denisko et al. (Tomsk) on the analysis of efficacy and cytokine profile in personalized cell therapy for endothelial-epithelial corneal dystrophy.

I.A. Stepanov and V.A. Beloborodov (Irkutsk) proved that the spinal fat index allows predicting the risk of developing septic spondylodiscitis after percutaneous laser decompression of the lumbar intervertebral discs, which is important to use in preoperative preparation.

A number of works are devoted to surgery of the gastrointestinal tract: P.V. Kolyadko et al. (Nizhnevartovsk, Omsk) described the use of a longitudinal resection of the stomach to reduce body weight and compensate for type 2 diabetes mellitus; article by V.A. Gankov et al. (Barnaul) is devoted to the surgical treatment of strangulated diaphragmatic hernias.

One of the few experimental works in this issue is an article by S.V. Notova et al. (Orenburg) devoted to the actual problem of the emerging imbalance of microelement composition, including zinc, in the blood and liver of rats with a high-calorie diet.

Rare for our journal (unfortunately) is an important article for healthcare organizers from Barnaul (Kolyado E.V. et al.) on the use of digital technologies and the creation of a system for working with undesirable events and making management decisions to eliminate and prevent them.

As always, this issue contains interesting reviews and meta-analyses on various clinical and historical issues presented by authors from Irkutsk, Kurgan, Tver, Volgograd and Yekaterinburg.

Many thanks to the authors for the work done and interest in our journal, and to the reviewers for qualified reviews in a short time.

Thanks to you, the journal is becoming better and more popular, as evidenced by the attendance of its website.

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ПРЕДИСЛОВИЕ ГЛАВНОГО РЕДАКТОРА К № 1 (2023)

Колесников Сергей Иванович

академик РАН

Уважаемые читатели!

Изучив статьи, представленные в данном номере, мне не удалось выделить одну передовую статью, т. к. большая часть опубликованных работ – это работы приблизительно одного, высокого, уровня. Поэтому я остановлюсь на тех статьях, которые могут вызвать интерес не только у узких специалистов, но и у более широкого круга читателей.

Начну со статей, имеющих отношение к инфекционной патологии, которая так и не ушла с передовиц как научных журналов, так и популярных изданий. Это касается как COVID-19 и его последствий, так и вновь угрожающего населению гриппа A/H1N1.

Прежде всего это статья Д.Д. Брюховой и соавт. (Иркутск) о динамике гуморального иммунитета у детей в период распространения COVID-19. Они доказали высокую интенсивность скрыто протекающего эпидемического процесса и формирование относительно устойчивого иммунитета, что, на мой взгляд, требует отдельного обсуждения в плане предполагаемой вакцинации детского населения. В работе Т.И. Петелиной и соавт. (Тюмень) показано, что характер развития как психологических, так и сердечно-сосудистых осложнений после COVID-19 определяется пролонгированной сосудистой реакцией, нарушением реологических и метаболических свойств крови.

Естественно, что распространение инфекций требует применения методов оценки их возможной динамики, формирования иммунитета и коррекции возникающих нарушений. Статья А.В. Малярчикова и К.Г. Шаповалова (Чита) посвящена увеличенному риску развития органной недостаточности при тяжёлой пневмонии с полиморфизмом гена *TLR4* Asp299Gly при гриппе A/H1N1. А в работе В.Ю. Малыгиной и соавт. (Симферополь) выявлено, что введение сапонина таурозида Sx1 положительно влияет на функциональную активность селезёнки при инфицировании мышей вирусом гриппа H1N1.

В обзоре А.М. Зиганшина и соавт. (Уфа, Чита) доказывается перспективность применения технологии CRISPR-Cas9 в профилактике и лечении некоторых вирусных заболеваний. Статья Е.Ю. Зяблицкой с соавторами-учёными и производственниками из Симферополя и Москвы показала эффективность использования метода ELISPOT «ТиграТест® SARS-CoV-2» для выявления скрытых форм коронавирусной инфекции.

Не уходит из научной повестки нашей страны и проблема туберкулёза, в том числе при его коморбидности с ВИЧ-инфекцией, чему посвящена статья Е.А. Бородулиной и соавт. (Самара). Есть проблема и с диагностикой псевдотуберкулёза и его вспышек, о чём свидетельствует статья Т.Ю. Загоскиной и соавт. (Иркутск) по использованию дот-иммуноанализа для экспресс-идентификации культур *Y. pseudotuberculosis.*

Мне кажется, что внимание читателей привлечёт статья Н.Б. Семёновой и соавт. (Красноярск, Новосибирск) по распространённости психосоматической коморбидности расстройств пищевого поведения и особенно подпороговых пищевых расстройств у школьниц 11–17 лет. Особенностью клинических проявлений является частое отсутствие дефицита массы тела и коморбидность с болевым синдромом. Надеюсь, что вас заинтересует и статья Е.А. Горобец и О.Р. Есина (Казань) относительно связи уровня алекситимии у подростков с различными соматическими заболеваниями, что следует учитывать при оценке уровня алекситимии и центральной сенситизации у подростков с головными болями.

Несколько статей посвящены разработке и использованию методов нелекарственного воздействия при различных заболеваниях и их моделировании. Это работы Н.С. Трибрат и соавт. (Симферополь, Казань) по влиянию глюкозо-электролитного напитка с растительными антиоксидантами на показатели артерий вертебробазилярного бассейна после ступенчато-возрастающей нагрузки. Статья А.Э. Шобоева и соавт. (Бохан, Иркутск) доказывает, что метод рефлексотерапии в сравнении с физиотерапией более эффективен для восстановления функции глотания в постинсультном периоде.

Несколько статей посвящены хирургическим проблемам и их экспериментальному моделированию. В работе М.М. Бикбова и соавт. (Уфа) показана эффективность дозированной мышечно-конъюнктивальной резекции хряща верхнего века у пациентов с частичным птозом верхнего века. Также я бы выделил работу М.С. Дениско и соавт. (Томск) по анализу эффективности и цитокинового профиля при персонализированной клеточной терапии эндотелиально-эпителиальной дистрофии роговицы.

И.А. Степанов и В.А. Белобородов (Иркутск) доказали, что спинальный жировой индекс позволяет прогнозировать риск развития септического спондилодисцита после выполнения чрескожной лазерной декомпрессии поясничных межпозвонковых дисков, что важно использовать в предоперационной подготовке.

Ряд работ посвящены хирургии желудочно-кишечного тракта: П.В. Колядко и соавт. (Нижневартовск, Омск) описали использование продольной резекции желудка для снижения массы тела и компенсации сахарного диабета 2-го типа; статья В.А. Ганкова и соавт. (Барнаул) посвящена оперативному лечению ущемлённых диафрагмальных грыж.

Одна из немногих экспериментальных работ этого выпуска – статья С.В. Нотовой и соавт. (Оренбург) – посвящена актуальной проблеме возникающего дисбаланса микроэлементного состава, в т. ч. цинка, в крови и печени крыс при высококалорийной диете.

Редкой (к сожалению) для нашего журнала является важная для организаторов здравоохранения статья авторов из Барнаула (Колядо Е.В. и соавт.) по применению цифровых технологий и созданию системы работы с нежелательными событиями и принятия управленческих решений по их устранению и предупреждению.

Как всегда, в выпуске есть интересные обзоры и метаанализы по различным клиническим и историческим вопросам, представленные авторами из Иркутска, Кургана, Твери, Волгограда и Екатеринбурга.

Большое спасибо авторам за проделанную работу и интерес к нашему журналу, а рецензентам – за квалифицированные рецензии в сжатые сроки.

Благодаря Вам журнал становится лучше и популярнее, о чём свидетельствует посещаемость его сайта.

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CONTENTS

DISCUSSION PAPERS, LECTURES, NEW TRENDS IN MEDICAL SCIENCE

CELL-MEDIATED AND HUMORAL IMMUNITY DURING COVID-19 IN THE REPUBLIC OF CRIMEA

Zyablitskaya E.Yu. [1],
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ABSTRACT

The COVID-19 (coronavirus disease 2019) pandemic has spurred the development of highly effective quantitative methods for assessing the adaptive immune response to the SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2) virus. In order to assess the humoral component of the immune response, various methods for detecting immunoglobulins A, M, G are widely used. ELISPOT seems to be the most accessible and effective method to assess the level of T cells that specifically respond to the SARS-CoV-2 virus antigens.

The aim. *To assess cell-mediated and humoral immunity in COVID-19 in residents of the Republic of Crimea.*

Methods. *The study was performed on 24 volunteers: the presence of coronavirus antibodies was determined by ELISA method, and the presence of contact with coronavirus proteins – by the ELISPOT "TigraTest® SARS-CoV-2" method (Generium, Russia). For retrospective study of humoral immunity in the population, we assessed 10 000 ELISA tests (ECOlab IgM and IgG, Russia) performed in our laboratory for the period from July 2020 to January 2022.*

Results. *The results show the effectiveness of using the ELISPOT method to detect latent forms of coronavirus infection. It is important to note that there is statistically significant relationship between the timing of the disease and the number of spots in both antigen panels. After vaccination against SARS-CoV-2, cell-mediated immunity lasts up to 6 months or more.*

Conclusions. *As a result of the study, it was found that during 2021, the level of immunization of the population of the Republic of Crimea against COVID-19 has significantly increased; the proportion of residents who have positive IgG test has increased from 27 to 87 %. The results of ELISPOT studies using a set of reagents for in vitro detection of blood T-lymphocytes that specifically respond to SARS-CoV-2 virus antigens ("TigraTest® SARS-CoV-2") showed that this method is more sensitive than ELISA in detecting latent diseases.*

Key words: *cell-mediated immunity, COVID-19, SARS-CoV-2 ELISPOT, TigraTest*

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КЛЕТОЧНЫЙ И ГУМОРАЛЬНЫЙ ИММУНИТЕТ ПРИ COVID-19 В РЕСПУБЛИКЕ КРЫМ

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РЕЗЮМЕ

Пандемия COVID-19 (coronavirus disease 2019) послужила стимулом к разработке высокоэффективных количественных методов оценки адаптивного иммунного ответа на вирус SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2).

С целью оценки гуморального звена иммунного ответа широко применяют различные методы детекции иммуноглобулинов классов A, M, G. Для оценки уровня Т-клеток, специфически отвечающих на антигены вируса SARS-CoV-2, наиболее доступным и эффективным методом представляется ELISPOT.

Цель работы. *Оценить клеточный и гуморальный иммунитет при COVID-19 у жителей Республики Крым.*

Методы. *Выполнено исследование на 24 добровольцах: определяли наличие антител к коронавирусу методом иммуноферментного анализа (ИФА) и наличие контакта с белками коронавируса методом ELISPOT «ТиграТест® SARS-CoV-2» (АО «Генериум», Россия). Для ретроспективного исследования гуморального иммунитета в популяции оценили 10 000 ИФА-тестов (ЗАО «ЭКОлаб» IgM и IgG, Россия), выполненных в нашей лаборатории за период с июля 2020 по январь 2022 г.*

Результаты. *Полученные результаты показывают эффективность использования метода ELISPOT для выявления скрытых форм коронавирусной инфекции. При этом следует отметить, что есть статистически значимая связь между сроками заболевания и количеством спотов в обеих панелях антигенов. После вакцинации против COVID-19 клеточный иммунитет сохраняется до 6 месяцев и более.*

Выводы. *В результате исследования установлено, что на протяжении 2021 г. уровень иммунизации населения Республики Крым против COVID-19 существенно повысился; возросла доля жителей, имеющих положительный тест на IgG, – с 27 % до 87 %. Результаты исследований методом ELISPOT с использованием набора реагентов для выявления in vitro в крови Т-лимфоцитов, специфически отвечающих на антигены вируса SARS-CoV-2 («ТиграТест® SARS-CoV-2»), показали, что данная методика является более чувствительной, чем метод ИФА, способна выявлять перенесённые в скрытой форме заболевания.*

Ключевые слова: *клеточный иммунитет, COVID-19, SARS-CoV-2 ELISPOT, ТиграТест*

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INTRODUCTION

The relevance of studying the immune response during the COVID-19 pandemic (coronavirus disease 2019) lies in the value of predicting the possibility of disease and the severity of the disease to determine the timing of vaccination in humans, depending on the presence of specific immunity to SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2), and it is critical for epidemiological population-based prognostic studies.

The structural and molecular characteristics of SARS-CoV-2, as well as the stages of adaptive immune response, were the basis for the development of various laboratory diagnostic methods for assessing immunity in COVID-19 [1]. As a result of the past COVID-19, an immune system is formed with a simplified structure that includes: 1) immunoglobulins (Ig) of classes A, G, M; 2) SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cells; 3) B cells [2]. To study specific immunity, the most common methods are the detection of specific antibodies and the detection of activated T cells. The basic objects of the study were SARS-CoV-2-specific antibodies IgA, IgM IgG, determined by enzyme immunoassay (ELISA), as well as T cells synthesizing interferon γ (IFN-γ) in response to antigens of the SARS-CoV-2 virus [3, 4].

With the spread of the pandemic, a rapid evolution of ELISA methods was observed – from qualitative screening of total IgA, IgM, IgG antibodies against the virus up to quantitative detection of neutralizing IgG antibodies, including detection of the receptor-binding domain (RBD, receptor-binding domain) of the SARS-CoV-2 protein [5]. Later, for research purposes, the PNA (pseudovirus neutralization assay) method was used to assess the neutralizing ability of serum, when the SARS-CoV-2 pseudovirus infects cells expressing the ACE2 receptor, and after incubation with the tested serum, the degree of neutralization of the pseudovirus is calculated based on the luminescence value [6].

Three methods are relevant for the study of the cellular immune response: flow cytometry (by the proliferative response of T helpers ($CD4^+$) and killer T cells ($CD8^+$) to antigen restimulation *in vitro*); IGRA-ELISPOT (interferon-gamma release assay) (by the number of IFN-γ antigen-specific T cells producing among peripheral blood mononuclear cells (PBMC) and ELISA (by changing the concentration of IFN-γ in response to stimulation of T cells by pathogen antigens) [5].

Large studies described in the literature indicate that on the 21st day from the onset of the disease, the plasma of about 30 % of people who have past COVID-19 has low titers of neutralizing antibodies specific to SARS-CoV-2, or does not contain them at all [7, 8]. COVID-19 causes a pronounced T cell response lasting up to 15 months [9], T cells are widely produced in response to infection and vaccination [10], and IGRA-ELISPOT-based test systems detect 51 % more COVID-19 survivors than IgG ELISA tests [11]. As a consequence, SARS-CoV-2-specific T cells may be a more sensitive marker of the past COVID-19, and their detection methods complement serology in the complex laboratory diagnostics of the immune response to SARS-CoV-2.

The Republic of Crimea was isolated from the mainland during the pandemic period outside the holiday season in 2020 and 2021 due to travel restrictions. From May to September, however, there was a dramatic change in the epidemiological situation due to the active seasonal migration of people to the resort region, this being reflected in the specifics of the population immunity, which this study focuses on in comparing methods for determining the cellular and humoral immune response.

PURPOSE

To assess cell-mediated and humoral immunity in COVID-19 in residents of the Republic of Crimea.

METHODS

A study involving 24 volunteers (university staff) – 10 men and 14 women – with a known history of COVID-19 and vaccination was carried out in the summer of 2021 with the purpose of comparative evaluation of laboratory methods for diagnosis of immune response. Volunteers gave informed consent for the study and had their venous blood taken in two sealed test tubes (with sodium citrate coagulant) at the university clinic. The analysis for the presence of humoral and T cell immunity by ELISA and ELISPOT methods was carried out in the Central Research Laboratory. The inclusion criteria were age 20–40 years; absence of any disease in the acute phase. The study was carried out according to the instructions of the reagent kits: 1) ECOlab, CJSC IgM and IgG for the detection of immunoglobulins to various components of the SARS-CoV-2 coronavirus, including post-vaccination antibodies to S protein by ELISA; 2) TigraTest® SARS-CoV-2 (Generium, JSC) for *in vitro* detection of T-lymphocytes specifically responding to SARS-CoV-2 virus antigens. This is a version of the ELISA method IGRA ELISPOT (Interferon Gamma Release Assay, Enzyme-Linked Spot analysis), in which the cytokine interferon-gamma (IFNγ) binds to the surface of the culture plate membrane next to the secreting cells on the one hand and IFNγ binds to other antibodies conjugated to alkaline phosphatase on the other. Treatment with a chromogenic substrate, which is converted by alkaline phosphatase into a colored spot of insoluble precipitate at the reaction site, makes it possible to see the reaction. Each spot an imprint of a single T cell secreting IFNγ in response to contact with the virus antigen, and spot counts quantify the content of SARS-COV-2 antigen-specific $CD4^+$ and $CD8^+$ T cells in the blood. The result of the analysis is the calculation of the number of spots in the wells with controls and antigens.

For the study of humoral immunity in the population, 10,000 ELISA tests (ECOlab, CJSC IgM and IgG) performed in our laboratory for the period from July 2020 to January 2022 for the residents of Crimea without signs of respiratory disease who applied to verify previously past COVID-19 in asymptomatic form or without polymerase chain reaction

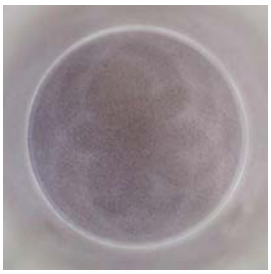
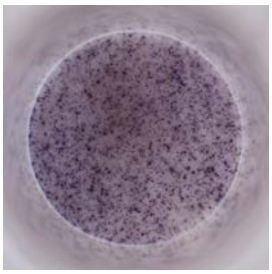
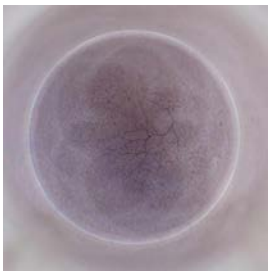
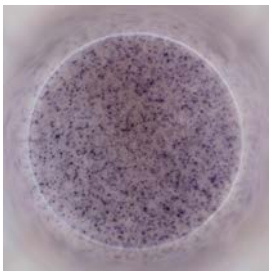
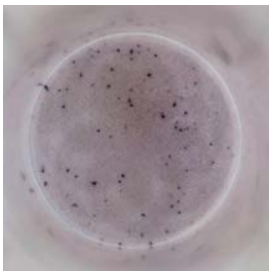
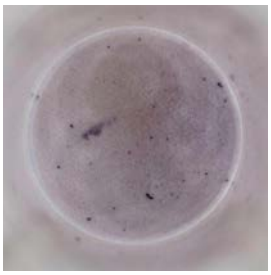
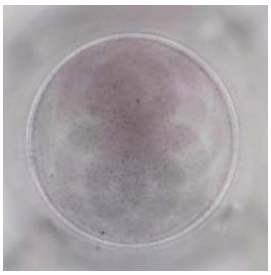
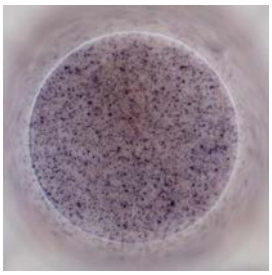
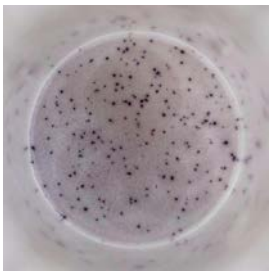
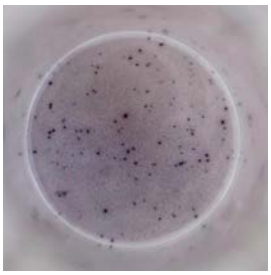
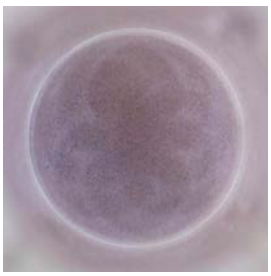
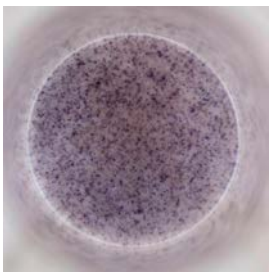
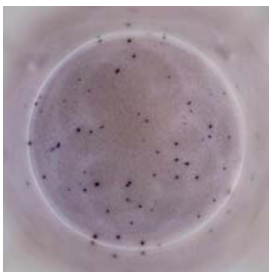
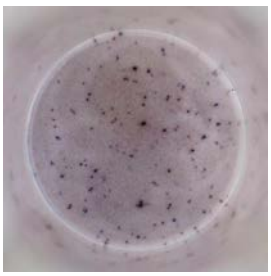
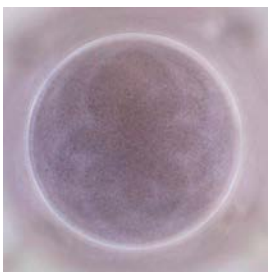
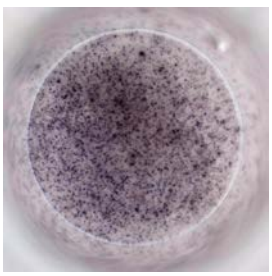
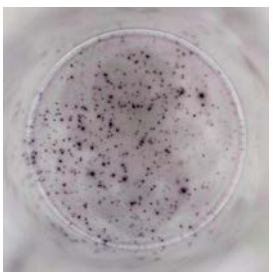
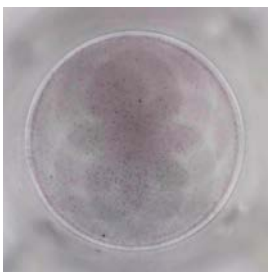
Patient	Negative control	Positive control	Antigen panel No. 1 (S protein peptides)	Antigen panel No. 2 (protein peptides N, M, O3, O7)
Never had a disease, not vaccinated				
	2	> 100	1	1
Had a disease 6 months before the study, vaccinated 1 month before the study				
	0	> 100	35	10
Vaccinated 4 months prior to the study, had a disease 2 months prior to the study				
	0	> 100	75	25
Not vaccinated, had a disease 3 months prior to the study				
	0	> 100	31	40
Never had a disease, vaccinated 3 months prior to the study				
	0	> 100	53	1

FIG. 1.

The results of studies on the example of patients from different groups: photographs of wells after incubation of lymphocytes with antigens and visualization of cells activated for the interferon production with color marks; magnification 20×

(PCR) confirmation as part of vaccination planning or evaluation of its effectiveness. All adult patients who contacted the laboratory to assess specific immunity to SARS-CoV-2 were included in the study. The mean age of the patients was 38 ± 9.8 years in all study periods. The male to female ratio was 4:5.

Statistical processing was performed using Statistica 10.0 (StatSoft Inc., USA). The Shapiro–Wilk method was used to determine the normality of the distribution of the trait: the number of spots in wells with antigen to peptide of protein S (AG1) and with antigen to peptides of proteins N, M, O3, O7 (AG2) when assessing cellular immunity. Differences between groups of patients (group 1 – not ill, not vaccinated; group 2 – not ill, vaccinated; group 3 – ill, not vaccinated; group 4 – ill, and then vaccinated) were evaluated by the Kruskall – Wallis test. The influence of factors on the number of spots in wells with antigens was evaluated by the ANOVA method. Controlled factors were the presence and timing of the disease and vaccination (from the medical history). Differences at $p \leq 0.05$ were considered statistically significant.

Ethical standards were observed in the work; participants signed a voluntary informed consent, and the work was approved by the ethics Committee of the V.I. Vernadsky Crimean Federal University (Protocol No. 4 dated 12.04.2022).

RESULTS AND DISCUSSION

In course of the study of cellular immunity, according to the medical history, the studied individuals were distributed as follows: group 1 (not ill, not vaccinated) – 12 % (n = 3); group 2 (not ill, vaccinated) – 21% (n = 5); group 3 (ill, not vaccinated) – 38% (n = 9); group 4 (ill, and then vaccinated) – 29% (n = 7).

Significantly, the study was performed 12 months after the onset of the pandemic in Crimea, and antibody levels were 100% reflective of a 6-month medical history; longer periods after the disease were not investigated. That is, all individuals of the group 1 had a negative antibody level, and those of the groups 2–4 had a positive level of IgM or IgG detected only in the case of a previous disease (with PCR confirmation) or vaccination during the last six months. In the case of disease or vaccination at an earlier period, individuals in groups 2, 3, 4 had negative antibody levels. For the uniformity of the study, only those vaccinated with the two components of the Sputnik V vaccine were included in the number of vaccinated volunteers.

As a result of the ELISPOT study, according to his interpretation, the groups were redistributed: group 1 – 4 % (n = 1); group 2 – 17 % (n = 4); group 3 – 46 % (n = 11); group 4 – 33 % (n = 8). Only 1 person had no cellular immunity, the rest (healthy according to medical history and laboratory tests for the presence of antibodies to coronavirus) showed spots in wells with AG1 and AG2 in levels which were evidence of subclinical disease. Among those in group 2, 1 out of 5 individuals had spots in the AG2 panel, indicating a latent post-vaccination disease. Among those in groups 3 and 4, there was a perfect match between the results of the study and the medical history, but these groups increased by individuals who had previously formed groups 1 and 2 (based on the medical history and antibody levels). However, it should be noted that there is a statistically significant association between the timing of the disease and the number of spots in both panels of antigens in group 3 and in panel AG2 in group 4 patients. So, the more time passed since recovery, the fewer activated T-cells were detected. The number of spots in the panel of antigens against S-protein in vaccinated individuals ranged from 35 to 75, even 6 months after vaccination (Fig. 1).

The statistical results are shown in Figures 2 and 3. A comparison of the number of spots in the AG1 and AG2 wells between groups has revealed that the number of spots in the AG1 well was statistically significantly higher in the vaccinated group than in those in groups 1 and 3 who did not receive the vaccine. Individuals from group 3 who had PCR-confirmed coronavirus infection had a statistically significantly higher number of spots in the wells with AG2 than those in the groups with no previous disease or latent disease. Univariate ANOVA test showed a statistically significant effect of the presence of the ailment and its timing on the number of spots in the well with AG2 (F = 12.40 and F = 7.88, respectively). Vaccine availability and timing had a statistically significant effect on the number of spots to both antigen panels (F = 21.98 for AG1 and F = 21.01 for AG2). However, it is worth noting that the number of spots in the well with AG1 to S protein is statistically significantly higher, while the number in the well with AG2 to N, M, O3, O7 peptides is statistically significantly lower than in the absence of vaccination. The timing of the vaccine has a similar effect, but its degree is much lower (F = 10.10 for AG1 and F = 5.05 for AG2).

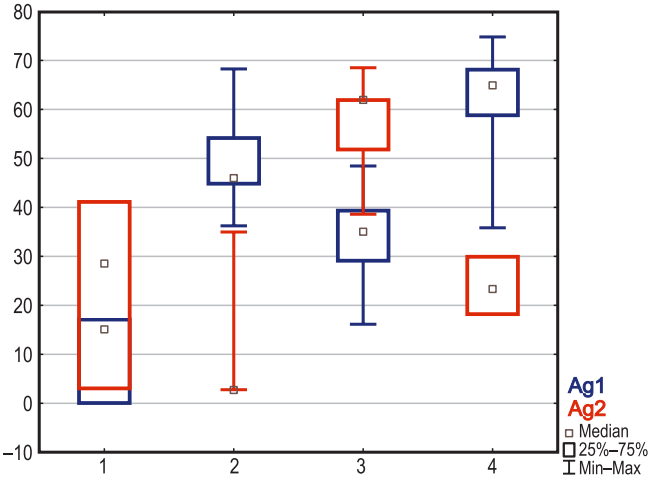


FIG. 2. *Mean values of the spots number in wells with antigens to S protein (AG1) and to N, M, Orf3a and Orf7a proteins (AG2)*

The study of humoral immunity has shown a progressive increase in immunisation in the population of the Republic of Crimea. Only 27% of patients had a positive test for any class of antibodies (n = 4499) in the autumn-win-

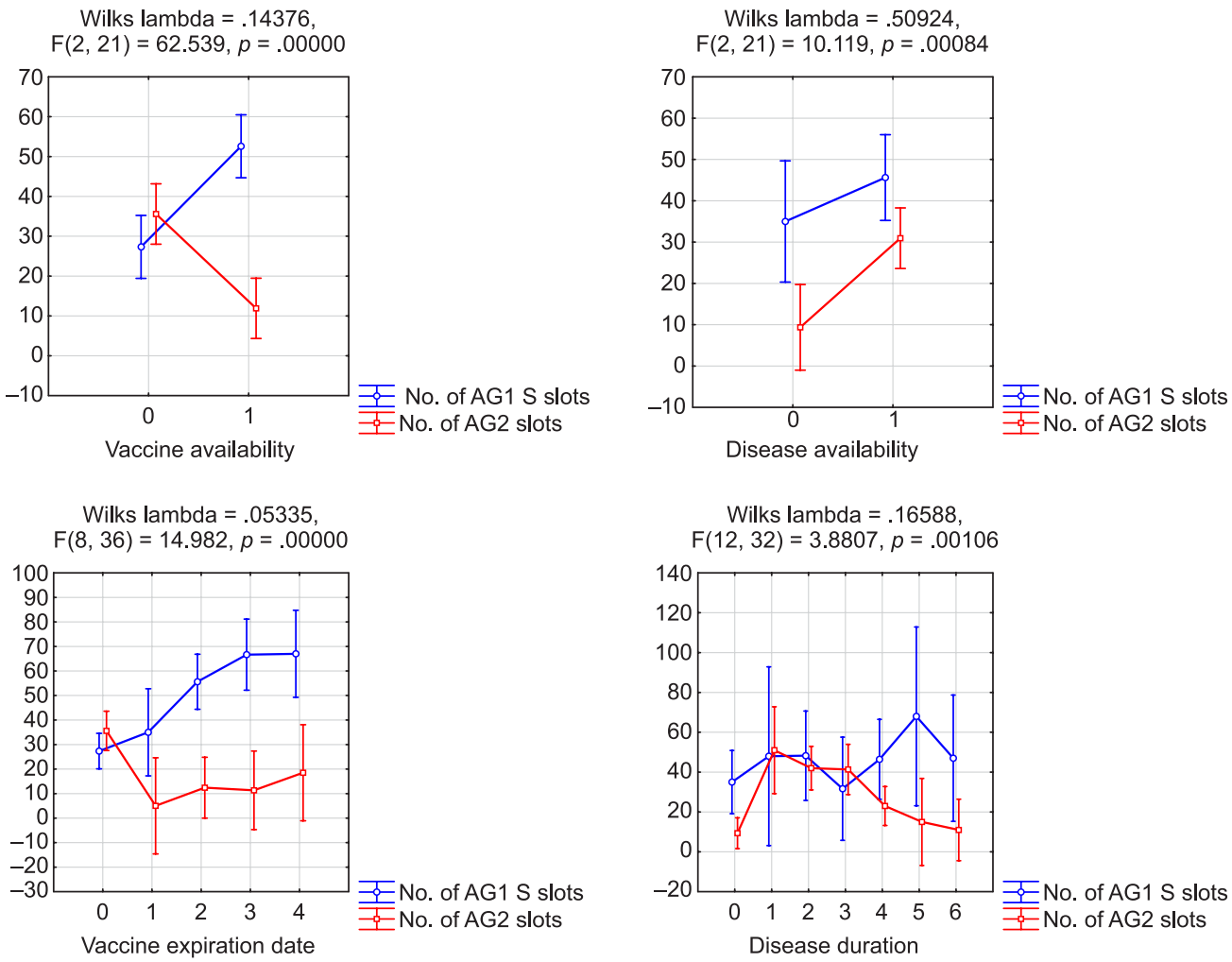


FIG. 3.

The degree of influence of various factors (the presence and timing of the disease, the presence and timing of vaccination) on the spots number (activated T cells) to AG1 and AG2 panels

ter period of 2020–2021, the majority having a predominance of IgG; the percentage of positive samples was 47 % in the spring of 2021(n = 1760) with a predominance of IgG and total post-vaccination antibodies. Positive sample proportions increased in the summer and autumn of 2021 to 61–63 % (n = 2286); it reached 87 % by the winter of 2021 2022. (n = 1455). The positive dynamics of these monitoring indicators in the Republic of Crimea is evidence of active immunization of the population, and the increase in the number of immunized individuals to 80–87% coincided with the sharp decline, in fact the cessation, of the epidemic wave in the region.

CONCLUSIONS

As a result of the study, it was found that the level of immunisation against COVID-19 in the Republic of Crimea has increased throughout 2021, with an increase in the proportion of residents who had a positive IgG test (a rise from 27 to 87 %). The results of ELISPOT studies using a set of reagents for *in vitro* detection of T lymphocytes in the blood that specifically respond to the antigens of the SARS-CoV-2 virus (TigraTest® SARS-CoV-2) showed that this technique is more sensitive than the ELISA method and can be used to diagnose latent disease.

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Conflict of Interest

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

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EATING DISORDERS IN 11–17 YEAR OLD SCHOOLGIRLS: PREVALENCE, FEATURES OF CLINICAL MANIFESTATIONS, PSYCHOSOMATIC COMORBIDITY

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ABSTRACT

Background. *Eating disorders are an urgent public health problem due to their high prevalence and mortality. The disease prognosis depends on timely diagnosis; however, these conditions are sure to be underestimated.*
The aim. *To study the prevalence, features of clinical manifestations and psychosomatic comorbidity of eating disorder (ED) and subthreshold eating disorder (SED) in schoolgirls aged 11–17 years.*
Material and methods. *We examined 917 schoolgirls aged 11–17 years. The screening questionnaire including 11 questions combined into three pools named "Thoughts about one's own body" was used. The first pool (A) – assessed body dissatisfaction, the second one (B) – eating disorders, the third one (C) – food intake disorders. The answers were encoded as "1", "2", "3" ("false", "rather true", "true"). Schoolgirls who scored the maximum number of points (12) in the pool A were regarded as dissatisfied with their body and were further divided into two groups: the first group (ED) included girls who scored more than 10 points in the pool B; girls who scored less than 10 points were in the second group (SED), respectively. Body mass index (BMI) was determined by the weight-height coefficient correlated with centile tables. Psychosomatic comorbidity was assessed by the presence of recurring headache and abdominal pains in the last six months.*
Results. *The overall prevalence of eating disorders was 11.7 %, where EDs made 2.1 %, SEDs – 9.6 %. All schoolgirls had abnormal eating behavior; however, those with SEDs used less aggressive weight-loss methods. BMI < 5th percentile was observed in 10 % of girls with EDs and 4.5 % – with SEDs. 60 % of girls with ED and 40.9 % with SED complained about frequent headaches; 30 % of girls with ED and 20.4 % with SED were suffering from frequent abdominal pain.*
Conclusion. *In schoolgirls, subthreshold eating disorder is 4.6 times more common than threshold eating disorder. Characteristics of clinical manifestations are the absence of underweight for most schoolgirls and comorbidity with pain syndrome.*

Key words: *eating disorder, subthreshold eating disorder, schoolgirls, prevalence, pain syndrome*

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НАРУШЕНИЯ ПИЩЕВОГО ПОВЕДЕНИЯ У ШКОЛЬНИЦ 11–17 ЛЕТ: РАСПРОСТРАНЁННОСТЬ, ОСОБЕННОСТИ КЛИНИЧЕСКИХ ПРОЯВЛЕНИЙ, ПСИХОСОМАТИЧЕСКАЯ КОМОРБИДНОСТЬ

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РЕЗЮМЕ

Обоснование. *Нарушения пищевого поведения являются актуальной проблемой здравоохранения, однако в детско-подростковом возрасте эти состояния часто недооцениваются.*
Цель. *Изучить распространённость, особенности клинических проявлений и психосоматическую коморбидность расстройств пищевого поведения (РПП) и подпороговых пищевых расстройств (ППР) у школьниц 11–17 лет.*
Материал и методы. *Обследовано 917 школьниц в возрасте 11–17 лет. Применялся опросник «Мысли о собственном теле», состоящий из трёх блоков. Первый блок (А) оценивал неудовлетворённость телом, второй блок (В) – нарушения пищевого поведения, третий блок (С) – нарушения приёма пищи. Ответы кодировались как «1», «2», «3» («не верно», «отчасти верно», «верно»). Девочки и девушки, набравшие 12 баллов в сумме ответов на блок А, расценивались как неудовлетворённые своим телом и далее делились на две группы в зависимости от результатов ответа на блок В: в группу РПП отнесены школьницы, набравшие более 10 баллов; в группу ППР – набравшие менее 10 баллов. Индекс массы тела определялся по весо-ростовому коэффициенту. Психосоматическая коморбидность оценивалась по наличию повторяющейся головной и абдоминальной боли.*
Результаты. *Общая распространённость нарушений пищевого поведения составила 11,7 %, из них: РПП – 2,1 %; ППР – 9,6 %. В обеих группах отмечалось аномальное пищевое поведение, но школьницы с ППР использовали менее агрессивные методы снижения веса. ИМТ < 5-го перцентиля имели 10 % школьниц с РПП и 4,5 % – с ППР. Жалобы на головные боли предъявляли 60 % школьниц с РПП и 40,9 % – с ППР; на боли в животе – 30 % школьниц с РПП и 20,4 % – с ППР.*
Заключение. *В детско-подростковом возрасте подпороговые пищевые расстройства встречаются в 4,6 раза чаще, чем пороговые. Особенностью клинических проявлений является частое отсутствие дефицита массы тела и коморбидность с болевым синдромом.*

Ключевые слова: *расстройства пищевого поведения, подпороговые пищевые расстройства, распространённость, болевой синдром, девочки, девушки, школьницы*

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OBJECTIVES

Eating disorders (EDs) are abnormal behavior associated with eating and preoccupation with food, which is accompanied by expressed concern about weight and figure. The main manifestations of EDs include anorexia nervosa (AN), bulimia nervosa (BN), and binge-eating disorder (BED). Risk factors for EDs are female gender, social factors (the presence of siblings, mass-media pressure), psychological (perfectionism, low self-esteem, negative emotions), and physical (overweight, slim parents) [1, 2]. Dissatisfaction with one's own body usually acts as a trigger mechanism of EDs [3–5]. Constant concern about the weight and (or) figure has a strong impact on self-esteem, leads to significant problems in important areas of functioning – personal, family, social, educational, professional. The prognosis of the disease is often unfavorable, which is explained by the persistent course of the disease, an indifferent attitude to one's health, ignoring medical appointments, and discontinuation of therapy [6–8].

The importance of EDs for public health is explained by their high prevalence, frequent comorbidity with other mental disorders [3, 9, 10], high mortality rates, mainly from complications from the cardiovascular system and suicide [11, 12]. The prevalence of AN, BN and BED among adults is 1–4 %, 1–2 % and 1–4 % [13]; among adolescents – 0.3 %, 0.9 % and 1.6 %, respectively [14]. However, the actual prevalence of EDs is much higher [2, 13], and, as studies show, no more than one third of patients are detected in the healthcare system [13].

Insufficient diagnosis of eating disorders in childhood and adolescence can be explained by the fact that many children and adolescents do not meet all the criteria of EDs, although they develop physical and psychological consequences caused by restrictions on food intake [15, 16]. Therefore, eating disorders in children and adolescents often have an atypical, latent nature, which can be regarded as subthreshold eating disorders (SED). Their significance is explained by their high prevalence in childhood and adolescence and the steady growth of relatively typical forms [15, 16, 17]. It is proved that the successful outcome of the ailment depends on timely diagnosis and early intervention [18, 19]. At the same time, the prevalence and features of clinical manifestations of eating disorders in children and adolescents remain poorly studied.

THE AIM OF THE STUDY

To study the prevalence, features of clinical manifestations and psychosomatic comorbidity of eating disorders and subthreshold eating disorders in schoolgirls aged 11–17.

METHODS

The study was conducted within the framework of the Eurasian Child & Adolescent Mental Health Study (EACMHS) [20]. Data collection was carried out in 2015–2018 in two major cities of Siberia – Novosibirsk and Krasnoyarsk. 11 secondary schools were surveyed in Novosibirsk, and 7 – in Krasnoyarsk. After receiving informed consent from parents, students were notified of the voluntary, anonymous and confidential nature of the study, and they were asked to fill out a questionnaire within an academic hour (45 minutes). The criteria for inclusion in the study were female gender, 11–17 years of age. The exclusion criteria were male gender and rejection of the study by parents or adolescent.

The continuous method was used to examine 917 girls aged 11–17 years (average age – 14.6 ± 1.3 years). Younger teenagers (11–13 years old) made up 168 subjects (18.3 %), middle (14–15 years old) – 468 subjects (51 %), older (16–17 years old) – 281 subjects (30.6 %).

A questionnaire developed as part of the EACMHS [1], which included several sections, was used in the study. Thoughts about your own body Section [21] allows the one to assess the presence of eating disorders and consists of 11 statements combined into three blocks. The first (A) block includes 4 statements revealing dissatisfaction with own body ("I am not satisfied with my body", "I am terrified of even a small weight gain", "I would like to be slimmer", "I am afraid to get fat"). The second (B) block includes 4 statements evaluating the symptoms of eating disorders ("I intentionally caused vomiting after eating", "I took medications to control my weight", "I train a lot to not gain weight", "I was on a diet"). The third (C) block includes 3 statements evaluating the symptoms of eating disorders ("I consume a large amount of food at a time", "I am not always able to control my food", "I have lost a lot of weight in a short time because I ate the wrong way"). There are 3 options of answers to each statement: "false", "partly true", "true", which were encoded as "1", "2", "3".

The responses were processed in two stages. At the first stage, the responses to the statements of block A were evaluated: schoolgirls who scored the maximum score in total (12 points) were regarded as dissatisfied with their bodies; schoolgirls who received a total of 4–11 points were assigned to the Control Group (CG). At the second stage, the symptoms of eating disorders in students dissatisfied with their bodies were assessed by the sum of the answers to the questions in block B: schoolgirls who received a total of 10 points or more were assigned to the ED group; the remaining adolescents were assigned to the SED group.

All schoolgirls had their body mass index (BMI) determined by the Quetelet index (weight expressed in kilograms)/height^2 (expressed in meters), with further interpretation according to the centile tables [22]. A percentile of less than 5 was interpreted as a weight deficit, from 5 to 85 – as a normal weight, from 86 to 95 – as overweight, over 95 – obesity.

Recurring pains were assessed by the frequency of a headache that interferes with concentration and the frequency of recurring abdominal pain over the past six months. There were 4 options of answers: "at least once a week", "at least once a month", "even less often", "almost never".

Ethical review

Approval for the study was received from the local Ethics Committee of the "Scientific Research Institute of Neurosciences and Medicine (minutes of the meeting No. 3 dated March 25, 2015). Anonymity of the study was achieved by filling out the questionnaire anonymously; by independently sealing one's questionnaire in a special envelope; by sealing all questionnaires in one common envelope.

Statistical analysis

Statistical processing of the obtained results was carried out using the IBM SPSS application software package, v. 22 (IBM Corp., USA). The type of distribution was determined using the Shapiro-Wilk test and Kolmogorov-Smirnov test. Statistical analysis of qualitative ordinal features was carried out by registering the number of objects in the sample having the same value of a qualitative variable, with further calculation of the relative frequency or proportion (%). The confidence interval (95% CI) for fractions and frequencies was estimated using the Wilson method. Comparison of groups by qualitative binary feature was carried out using the Pearson test χ^2 or Fisher's Exact Test (with the number of observations 5 or less). When describing statistical indicators, the absolute value of Pearson test χ^2 or Fisher's Exact Test, the number of degrees of freedom (df) for criterion χ^2 and the level of statistical significance of differences (p) were indicated. The level of statistical significance of the differences was established at $p = 0.05$, i. e. with an error probability of 5 %.

RESULTS

Prevalence of eating disorders

The overall prevalence of eating disorders was 11.7 %, of which ED – 2.1 %, SED – 9.6 % (Table 1).

In the age group of 11–13 years, the prevalence of disorders is the lowest and amounts to 1.6 %, and only subthreshold disorders are registered, while there are no clinically defined forms of eating disorders. At the age of 14–15 years, there is an increase in the prevalence of disorders up to 6.1 %, both due to an increase in SED by 3.2 times (up to 5.1 %) and due to the appearance of EDs (0.9 %). At the age of 16–17 years, there is a decrease in the overall prevalence of disorders to 4 % due to a decrease in subthreshold forms by 1.8 times (up to 2.8 %), while the threshold forms of ED increase to 1.2 %.

Features of clinical manifestations of eating disorders

Dissatisfaction with one's own body as the main symptom was noted in all schoolgirls with EDs. There was a fear of getting fat, an obsessive desire to lose weight, a desire to be slimmer, even a small weight gain was terrifying. 100 % of schoolgirls were on a diet, 95 % used medications to lose weight, 80 % caused vomiting, 45 % resorted to excessive sports or gymnastics (Fig. 1). The majority of girls and young women (75 %) noted that they lost a lot of weight in a short time because they "ate the wrong way". Symptoms of eating disorders in the form of consuming food in large quantities at a time were observed in 35 % of schoolgirls, binge-eating disorder – in 50 %. BMI analysis showed that the majority of schoolgirls (70 %) were of normal weight (5–85 percentiles), two subjects (10 %) had a body weight deficit (< 5th percentile), 4 subjects (20 %) were overweight (86–95 percentiles). There were no obese schoolgirls in the ED group (Fig. 2).

All girls and young women of this group had stable patterns of disturbed behavior aimed at avoiding food and striving for weight loss, which corresponded to the criteria of the ED. As for the specificity of clinical forms, 30 % should be considered of having BN, 10 % of schoolgirls – should be considered of having AN, and 15 % – should be considered of having BED. The remaining girls are classified as unspecified forms of EDs due to the discrepancy of BMI deficiency and/or the absence of such a clinical criterion as significant weight loss in a short time.

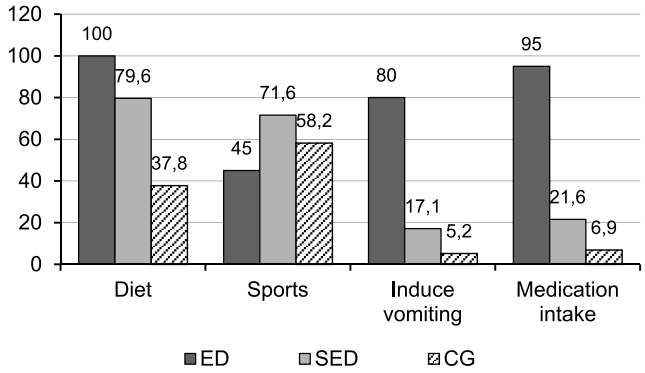

FIG. 1.

Frequency and methods of weight loss (%)

TABLE 1

PREVALENCE OF EATING DISORDER AND SUBTHRESHOLD EATING DISORDERS BY AGE (%, CI)

Age groups	Total	ED	SED
All ages (11–17 y. o.)	11.7 (9.8–14.0)	2.1 (1.4–3.3)	9.6 (7.8–11.7)
11–13 y. o.	1.6 (0.9–2.7)	0	1.6 (0.9–2.7)
14–15 y. o.	6.1 (4.7–7.8)	0.9 (0.5–1.8)	5.1 (3.9–6.7)
16–17 y. o.	4.0 (2.9–5.5)	1.2 (0.7–2.1)	2.8 (1.9–4.1)

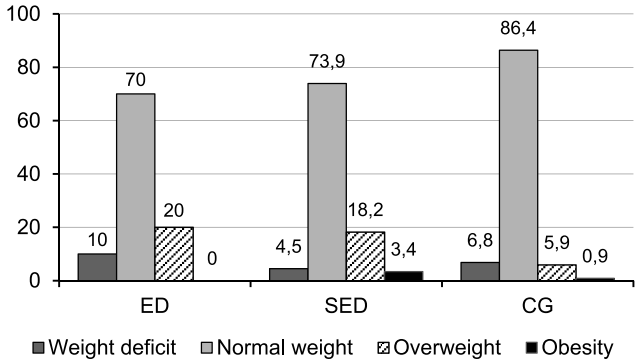

FIG. 2.
Distribution of respondents according to body weight (%)

Features of clinical manifestations of subthreshold eating disorders

All schoolgirls with SEDs were dissatisfied with their own body, the distorted image of which reached the level of super-valuable ideas, even a small weight gain caused horror. All girls and young women had symptoms of impaired eating behavior, but they differed in less severity and the use of less aggressive methods of combating excess weight, compared with the ED group (Fig. 2). Schoolgirls with SEDs were 4.7 times less likely to induce vomiting than schoolgirls with EDs (17.1 %; $\chi^2 = 38.1$; df = 2; $p < 0.001$), 3.4 times less likely to use medications (21.6 %; $\chi^2 = 47.7$; df = 2; $p < 0.001$), and less likely to resort to dieting (79.6 %; $\chi^2 = 11.8$; df = 2; $p = 0.003$). To reduce weight, the main emphasis was on sports (71.6 %; $\chi^2 = 14.1$; df = 2; $p = 0.001$). The result was not such a significant loss of body weight: only 11.3 % of schoolgirls said that they had lost weight significantly in a short time, and 40.9 % agreed with this only partially ($\chi^2 = 37.9$; df = 2; $p < 0.001$). Symptoms of eating disorders in the form of binge eating were noted with the same frequency as in the ED group (in 51.1 %; $p > 0.05$), and consuming large amounts of food at a time was 2 times less common (13.6 %; $\chi^2 = 6.1$; df = 2; $p = 0.05$). BMI analysis showed that the majority of girls and young women (73.9 %) were of normal weight, 4 schoolgirls (4.5 %) had a body weight deficit, 16 subjects (18.2 %) were overweight, three (3.4 %) were obese.

Association of eating disorders with recurring pain

Schoolgirls with eating disorders are more likely to complain of recurring pains, compared with the control group.

Thus, headaches are noted in 52.2 % of girls from the control group: 80% of schoolgirls with EDs and 70.4 % of schoolgirls with SEDs. At the same time, 60 % of schoolgirls with EDs and 40.9 % of schoolgirls with SEDs complain of frequent headaches (occurring every week for the last 6 months), which is 2 and 1.3 times more than girls and young women of the control group, respectively ($p = 0.003$; $\chi^2 = 20.21$; df = 6). The data obtained are shown in Figure 3.

Complaints of recurrent abdominal pains are presented by 46.6 % of schoolgirls in the control group, 65 % of schoolgirls with EDs and 56.8 % of girls and young women with SEDs. At the same time, frequent abdominal pains (occurring every week for the last 6 months) is noted by 30 % of schoolgirls with EDs and 20.4 % of schoolgirls with SEDs, which is 2.3 and 1.6 times more than in the control group (12.9 %).

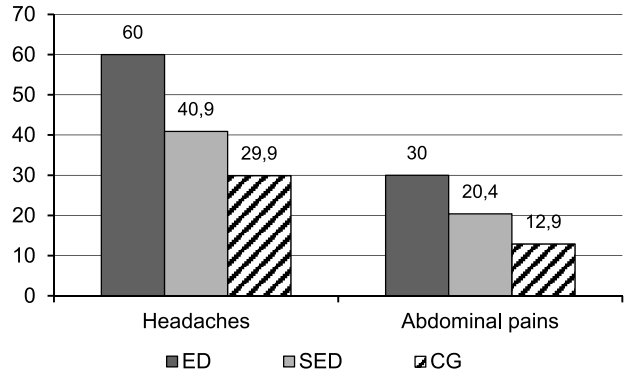

FIG. 3.
Frequent recurring pains (%)

DISCUSSION

Study main result summary

The overall prevalence of eating disorders in schoolgirls aged 11-17 years was 11.7 %, of which ED – 2.1 %, SED – 9.6 %. All schoolgirls had abnormal eating behavior, but girls and young women with SEDs used less aggressive methods of weight loss. BMI < 5th percentile was observed in 10 % of girls with EDs and 4.5 % – with SEDs. Complaints of frequent headaches were presented by 60 % of girls and young women with EDs and 40.9 % – with SEDs; for frequent abdominal pain – 30 % of girls and young women with EDs and 20.4 % – with SEDs.

Study main results discussion

According to the results of the study, the prevalence of eating disorders among schoolgirls aged 11–17 years is 11.7 %, subthreshold disorders predominate over threshold ones 4.5 times (9.6 and 2.1 %, respectively). Our data do not contradict the results obtained in European countries, where the threshold values of eating disorders were 2.9 %, and the subthreshold values were 11.5 % [23].

The age dynamics of the prevalence of eating disorders has shown that at the age of 11–13 years mainly subthreshold eating disorders are observed. At the age of 14–15 years, there is an increase in SED by 3.2 times, and clinically outlined forms of ED appear. At the age of 16–17 years, there is a decrease in subthreshold forms by 1.8 times, with a further increase in the threshold values of ED. Our data are consistent with the results of other authors, who confirm that subthreshold eating disorders are typical for adolescence, however, as they grow older, their frequency decreases, and only a small number of cases pass into adulthood [16]. This is due both to the physiological characteristics of adolescence and to the too narrow diagnostic framework of International Classifications of Diseases, according to which it is difficult to classify adoles-

cents into a certain group of ED, since they are often unable to formulate appropriate complaints due to insufficiently developed abstract thinking [24–26]. Therefore, unspecified, atypical or subthreshold types of eating disorders are more prevalent in adolescence [2], and the diagnostic transition, or crossover, is considered a common phenomenon and reflects the instability of ideas about a particular eating disorder [17, 19].

We have revealed that many schoolgirls, both with threshold and subthreshold values of ED, in the presence of a stable pattern of irregular eating behavior, there is no body weight deficit. At the same time, 18.5 % of schoolgirls have a BMI above the 85th percentile, which qualifies as excess weight, and 2.8 % have a BMI above the 95th, which is regarded as obesity. The data obtained by us are consistent with the opinion of other authors, who confirm that there may be no body weight deficit in eating disorders, and the BMI value does not always reflect the severity of the disease [27]. It has been shown that overweight adolescents are at risk for the development of EDs, and clinical manifestations of eating disorders in them have subthreshold values due to non-compliance of BMI with diagnostic criteria [28, 29].

Our study revealed that girls and young women with eating disorders are more likely than schoolgirls of the control group to complain of recurring pains, including headaches and abdominal pain. It is known that recurring pains in adolescents can occur simultaneously with EDs [30], and sometimes precede eating disorders [31]. Usually, such adolescents are initially diagnosed with abdominal pain, autonomic dysfunction or headache [32], which leads to late definition of the disease and worsens its course and prognosis. For example, recent catamnestic observations of children and adolescents discharged from the rheumatology department, where they were treated for chronic pain syndrome, showed that 22.4 % of young people were diagnosed with EDs in the future [30].

Therefore, internists in their practice should remember about eating disorders as one of the possible causes of pain in girls and young women, especially if the organic cause of pain is excluded. In this case, it is recommended to ask the question: *"Are you satisfied with your appearance?"* [27]. Upon receiving a negative answer, the doctor can conduct a short conversation, including the following questions: *"Would you like to become slimmer? Are you doing something for this? Have you been on a diet? Do you do a lot of sports? Do you control your weight with medication? Have you tried to intentionally induce vomiting?"*.

It should also be kept in mind that patients with EDs often dissimulate their condition. Therefore, doctors and parents are asked to evaluate the behavioral equivalents of symptoms indicating eating disorders: a teenager often measures his/her weight, evaluates his/her figure, expresses concern with his/her body verbally or in drawings [27]. Prodromal symptoms may also manifest as food restriction, episodes of overeating and vomiting ("purging"). If eating disorders are suspected, the patient should be referred to a psychotherapist to exclude the disease and start therapy in a timely manner, if necessary.

Study limitations

This study had a number of limitations. Firstly, conclusions about eating disorders were made on the basis of a screening examination, no further verification of the diagnosis was carried out, which reduces the diagnostic value of the results obtained. This is due to the fact that when planning the screening, we took into account the psychological characteristics of adolescence, and in order to obtain more valid answers, the survey was conducted anonymously. Secondly, this study was simultaneous, which reduces its predictive value and does not allow to trace the dynamics of clinical manifestations. Nevertheless, the conducted study provides a number of new knowledge that can be used in the practice of internists.

CONCLUSION

Thus, among schoolgirls aged 11–17, the prevalence of eating disorders is 11.7 %, most of them are represented by subthreshold values (9.6 %). The peculiarity of the clinical manifestations of EDs in childhood and adolescence is the absence of a body weight deficit in most children and adolescents in the presence of a stable pattern of impaired eating behavior.

Eating disorders in childhood and adolescence are often accompanied by pain syndrome, including recurrent abdominal pain (up to 65 %) and periodic headaches (up to 80 %), which makes such children and adolescents patients of somatic clinics. Therefore, internists need to remember about borderline conditions, one of which is eating disorders.

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Conflict of interest

The authors declare the absence of a conflict of interest.

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BIOCHEMISTRY

THE EFFECT OF A HIGH-CALORIE DIET ON THE TOTAL CONTENT OF CHEMICAL ELEMENTS AND METAL-LIGAND FORMS OF ZINC IN THE BLOOD SERUM AND LIVER OF WISTAR RATS

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ABSTRACT

Background. *Worldwide, there is a rapid increase in the number of people suffering from various forms of carbohydrate and lipid metabolism disorders. Modern studies show that the transport, distribution, excretion and accumulation of chemical elements in these types of metabolic disorders change in different ways and affect the further functional state of the body differently.*

The aim. *To evaluate the level of macro- and microelements in the blood serum and liver, as well as the content of metal-ligand forms of zinc in the blood serum of a Wistar rat in a high-calorie diet.*

Materials and methods. *Thirty male rats were selected for the experiment, from which two groups were formed: control (n = 15) and experimental (n = 15). The animals of the control group received the basic diet (270 kcal/100 g), and the animals of the experimental group received a high-calorie diet. During the experiment, the caloric content of the diet of the experimental group gradually increased from the caloric content of the total diet. During the study, body weight, biochemical parameters of blood and urine were evaluated. The analysis of macro- and microelements in the samples was carried out using inductively coupled plasma mass spectrometry. Determination of the content of individual zinc compounds in blood serum was carried out using a combination of a chromatograph and a mass spectrometer.*

Results. *It was found that a high-calorie diet led to a decrease in the level of iron, chromium, iodine, zinc, potassium, calcium, and an increase in vanadium in blood serum. In the liver, there was a decrease in the level of lithium and an increase in the level of calcium, vanadium, chromium, iron, zinc, cobalt. When assessing the chemical forms of zinc in the blood serum, a percentage increase in the albumin fraction was recorded against the background of a decrease in amino acid complexes and low-molecular-weight forms of zinc.*

Conclusion. *The data obtained suggest that a high-calorie diet leads to an imbalance of chemical elements, which can serve as one of the triggers for dysregulation of a number of physiological functions of the body.*

Key words: *trace elements, zinc, high-calorie diet, overweight, obesity, speciation analysis*

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ВЛИЯНИЕ ВЫСОКОКАЛОРИЙНОЙ ДИЕТЫ НА СОДЕРЖАНИЕ ХИМИЧЕСКИХ ЭЛЕМЕНТОВ И МЕТАЛЛО-ЛИГАНДНЫЕ ФОРМЫ ЦИНКА В СЫВОРОТКЕ КРОВИ И ПЕЧЕНИ КРЫС ЛИНИИ WISTAR

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РЕЗЮМЕ

Обоснование. *Во всём мире отмечается стремительный рост числа людей, страдающих различными формами нарушения углеводного и липидного обмена. Современные исследования показывают, что транспорт, распределение, экскреция и накопление химических элементов при данных видах нарушения обмена изменяются по-разному и неодинаково влияют на дальнейшее состояние организма.*

Цель исследования. *Оценить уровень макро- и микроэлементов в сыворотке крови и печени, а также содержание металло-лигандных форм цинка в сыворотке крови у крыс линии Wistar в условиях высококалорийной диеты.*

Материалы и методы. *Для проведения эксперимента было отобрано 30 крыс-самцов, из которых были сформированы две группы: контрольная (n = 15) и опытная (n = 15). Животные контрольной группы получали основной рацион (270 ккал/100 г), а животные опытной группы – высококалорийную диету. В ходе эксперимента калорийность диеты опытной группы ступенчато увеличивалась от калорийности общего рациона. В ходе исследования оценивали массу тела, биохимические параметры крови и мочи. Анализ макро- и микроэлементов в образцах проводился с помощью метода масс-спектрометрии с индуктивно-связанной плазмой. Определение содержания индивидуальных соединений цинка в сыворотке крови проводился на комбинации хроматографа и масс-спектрометра.*

Результаты. *Установлено, что высококалорийная диета приводила к снижению уровня железа, хрома, йода, цинка, калия, кальция и увеличению содержания ванадия в сыворотке крови. В печени отмечалось снижение уровня лития и увеличение уровня кальция, ванадия, хрома, железа, цинка, кобальта. При оценке химических форм цинка в сыворотке крови фиксировалось процентное увеличение альбуминовой фракции на фоне снижения аминокислотных комплексов и низкомолекулярных форм цинка.*

Заключение. *Полученные данные позволяют предположить, что высококалорийная диета приводит к дисбалансу химических элементов, что может служить одним из пусковых механизмом дизрегуляции ряда физиологических функций организма.*

Ключевые слова: *микроэлементы, цинк, высококалорийная диета, избыточный вес, анализ содержания химических форм элементов*

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INTRODUCTION

Worldwide, there is a rapid increase in the number of people suffering from various forms of carbohydrate and lipid metabolism disorders [1, 2]. This group of population has an increased risk of developing obesity, type 2 diabetes mellitus, as well as a number of other diseases [3]. The ability of the body to effectively adapt metabolism depending on the needs and intake of nutrients is known as metabolic flexibility [4]. The basis for maintaining a normal metabolism of the body is a full diet. However, the diet of modern man is characterized by an unprecedented high level of consumption of high-calorie food [5]. Despite the increased caloric content of the diet, such a diet does not meet the recommended dietary requirements for the consumption of nutrients [6]. Improper nutrition in combination with physical inactivity reduces the metabolic flexibility of the body [7].

Fundamental and clinical studies show that the transport, distribution, excretion and accumulation of chemical elements in various forms of carbohydrate and lipid metabolism disorders change differently and affect the further state of the body differently [8, 9]. This is due to the fact that elemental homeostasis is a particular form of the general homeostatic system of the body, changes in which lead to a disorder of the molecular mechanisms of adaptation [10]. Until recently, elemental homeostasis was assessed by the total content of chemical elements in various bio substrates. However, recent studies have shown that changes in the level of trace elements, even in the range of normal values, are accompanied by their redistribution in different fractions [11]. Thus, metabolic disorders can occur not only as a result of a deficiency or excess of a certain element, but also due to the interaction between various metal ions and the presence of metal-binding (chelating) agents [12, 13]. Modern studies confirm that the change in the ratios of the forms of chemical elements in biological systems is the main trigger mechanism for the regulation or dysregulation of many physiological functions of living organisms [14, 15]. Zinc deficiency is known to be a risk factor for obesity and diabetes. The study of zinc content is important because various forms of this trace element are involved in a variety of biochemical and physiological processes, including the transport of Zn^{2+} to the liver and other organs; participation in the processes of cytotoxicity and inflammation, deactivation of free radicals, etc. However, the vast majority of works are focused only on determining the total level of chemical elements in individual bio substrates, which does not allow for a detailed assessment of metabolism.

THE AIM OF THE STUDY

To evaluate the level of macro- and microelements in the blood serum and liver, as well as the content of metal-ligand forms of zinc in the blood serum of a Wistar rat in a high-calorie diet.

MATERIALS AND METHODS

The study was carried out on the basis of the experimental biological clinic (vivarium) of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. The experiment was performed on Wistar rats in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (National Standard of the Russian Federation GOST 33044-2014: Principles of Good Laboratory Practice). The design of the experiment was approved by the local Ethics Committee of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

Thirty male rats were selected for the experiment, from which two groups were formed: control (n = 15) and experimental (n = 15). At the beginning of the experiment, all laboratory animals were of the same age (12 weeks). The animals of the control group received the basal ration, and the animals of the experimental group received a high-calorie diet. The basal ration included a full-grain granular feed in accordance with GOST-R 50258-92 (full-grain feed for laboratory animals). The total caloric content of the ration was 270 kcal/100 g (20 % protein, 10 % fat and 70 % carbohydrates). A high-calorie diet was used to simulate the state of disorders of carbohydrate and lipid metabolism. The combination of a large amount of carbohydrates and fats of different origins more accurately mimics the human diet [16], in this regard, the diet is based on the addition of lard, coconut and sunflower oils to the overall balanced diet, a 10 % solution of fructose was used as a drink. All components of the diet were crushed into a homogeneous mixture, then granules were formed, which were subsequently dried in an oven at 25 °C. During the experiment, the caloric content of the diet gradually increased by 30 % (1–4 weeks), 60 % (5–8 weeks) and 90 % (9–12 weeks) of the caloric content of the total diet (Table 1). A step-by-step diet was used to gradually increase body weight in order to reduce the stress response of the body with an increase in caloric intake. The amount of feed consumed was 30 g per rat, which corresponds to the daily food requirement for this type of laboratory animals. Throughout the experiment, both in the experimental and control groups, complete feed consumption was recorded.

Every 4 weeks, an examination of animals was conducted, including an assessment of body weight, biochemical parameters of blood and urine, which made it possible to assess the disorder development degree of carbohydrate and lipid metabolism in the experiment. At the end of the test period, the animals were excluded from the experiment to collect biomaterial in order to assess the effect of a high-calorie diet on the body of laboratory animals.

Screening studies to assess the disorder development degree of carbohydrate and lipid metabolism were carried out by determining the level of glucose in an oral glucose tolerance test, total cholesterol, HDL, triglycerides and atherogenic index using a biochemical express analyzer Car-

TABLE 1
THE COMPOSITION OF A HIGH-CALORIE DIET (PER 100 G OF FEED)

Parameters	1–4 weeks	5–8 weeks	9–12 weeks
Caloric content, kcal	351	430	506
Proteins, %	17.2	14.5	12.1
Fats, %	20.8	32	42.3
Carbohydrates, %	62	53.5	45.6

dioChek (Polymer Technology System, USA). Urine analysis was performed using a semi-automatic analyzer Combilyzer 13 (Human, Germany) using Combina 13 test strips for the following indicators: color, volume, transparency, pH, specific gravity, erythrocytes, leukocytes, glucose, urobilinogen, protein, ketones, creatinine.

The analysis of macro- and microelements in blood serum and liver samples of laboratory animals was carried out using inductively coupled plasma mass spectrometry on an Elan 9000 (ICP-MS, PerkinElmer, USA). Determination of the content of individual zinc compounds in blood serum with high and low molecular weight biological ligands was carried out by high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-DRC-MS) on a combination of PerkinElmer S200 chromatograph (PerkinElmer, USA) and Elan 9000 mass spectrometer (PerkinElmer, USA).

The data obtained were processed by variation statistics methods using Statistica 10 statistical package (StatSoft Inc., USA). The hypothesis that the data belonged to a normal distribution was rejected in all cases with a probability of 95 %, which justified the use of non-parametric Mann – Whitney U test. The relationships between the parameters were evaluated using Spearman's Rank Correlation Coefficient. The correlation coefficients were estimated as follows: less than 0.3 – weak relationship, from 0.3 to 0.5 – moderate, from 0.5 to 0.7 – significant, from 0.7 to 0.9 – strong and more than 0.9 – very strong relationship. In all statistical analysis procedures, the achieved significance level (p) was calculated, while the critical significance level was assumed to be $p \leq 0.05$.

RESULTS

The results of the studies showed that with a step-by-step increase in the caloric content of the diet, the body weight of laboratory animals of the experimental group significantly increased relative to the control group (Fig. 1). By the 4th week of the experiment, the body weight of the animals of the experimental group increased by 9.8 %, by the 8th week – by 30 % and by the 12th week – by 44.6 %.

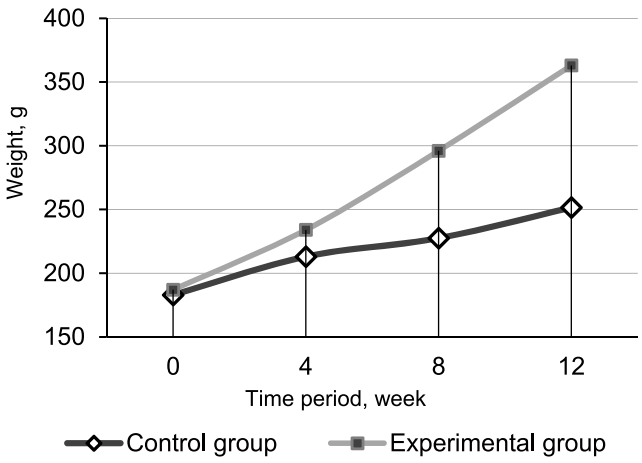

FIG. 1.
*The effect of a high-calorie diet on the dynamics of body weight of rats: ** – statistically significant difference between the experimental group and the control ($p \leq 0.01$)*

The results of a biochemical analysis of blood by the end of the study showed a statistically significant ($p \leq 0.01$) increase in glucose levels by 52.5 %, total cholesterol by 40.6 %, triglycerides by 127 %, an increase in the atherogenic index by 239 % and a decrease in HDL levels by 26.5 % in animals of the experimental group relative to the control group (Table 2). These changes were not spontaneous, but tended to change gradually, starting from the 4th week of the experiment.

The results of the oral glucose tolerance test by 12 weeks of the experiment showed significant differences between the experimental and control groups. Post-load glucose levels in the control group began to decrease after the 30th minute and by the 120th minute re-

turned to near baseline values; while in the experimental group the decrease started only after the 60th minute (Fig. 2).

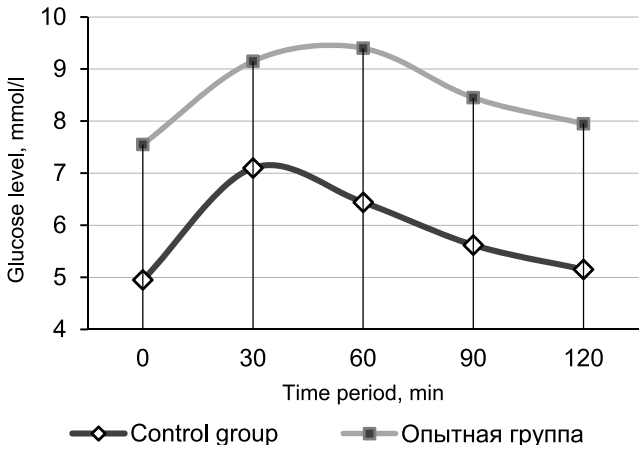

FIG. 2.

*The effect of a high-calorie diet on glucose levels in an oral glucose tolerance test (12 weeks of experiment): ** – statistically significant difference between the experimental group and the control ($p \leq 0.01$)*

The results of clinical and biochemical urinalysis did not undergo significant statistical changes at all stages of the experiment and were within normal values (Table 3).

Macro- and microelements are known to play an important role in the normal functioning of carbohydrate and lipid metabolism [17], so the content of chemical elements in the blood serum of laboratory animals was studied. In the experimental group relative to control, there was a statistically significant decrease in the level of iron (Fe) by 30 %, chromium (Cr) – by 20 %, iodine (I) – by 18 %, zinc (Zn) – by 11 %, potassium (K) – by 8.3 %, calcium (Ca) – by 6.3 % and an increase in vanadium (V) by 5.3 % (Fig. 3).

The liver is a key organ of homeostasis, the metabolic role of which consists in the metabolism of a number of nutrients, including chemical elements [18]. In this regard, the content of chemical elements in liver tissues was also evaluated. In the experimental group of rats, there was a statistically significant decrease in the level of lithium (Li) by 39.7 % and an increase in the level of Zn by 4.3 %, Ca by 32.4 %, V by 33.3 %, Cr by 45.2 %, Fe by 53.4 %, and cobalt (Co) by 74.4 % (Fig. 4).

During the correlation analysis between the chemical elements of blood serum and liver, the following pattern was established: in animals of the experimental group, as a result of high-calorie diet, a decrease in a number of chemical elements in blood serum was recorded, which led to their increase in the liver (iron, chromium, iodine, sodium, zinc, calcium) and vice versa (manganese). Strong negative correlation was observed for iron ($r = -0.728$), significant correlation – for chromium ($r = -0.654$) and calcium ($r = -0.679$), moderate correlation – for iodine ($r = -0.456$), weak correlation – for sodium ($r = -0.227$), zinc ($r = -0.171$) and manganese ($r = 0.116$). Lithium, selenium, boron, magnesium, potassium, phosphorus, copper, vanadium, and cobalt had the same tendency to change, both in blood serum and in the liver.

Zinc deficiency is known to be a risk factor for obesity and diabetes [19]. In this regard, the metal-ligand fractions of zinc in the blood serum of laboratory animals were evaluated. Four zinc-containing fractions were identified: α2-macroglobulin, albumin, amino acid complexes (AMC complexes) and low molecular weight forms of zinc. Figure 5 shows the results of the analysis of the percentage distribution of zinc by fractions when exposed to a high-calorie diet.

A statistically significant percentage increase in the albumin fraction from 45 to 66 % ($p < 0.05$) occurred in the experimental group of rats relative to the control affected by a decrease in AMC complexes and low molecular weight

TABLE 2

THE EFFECT OF A HIGH-CALORIE DIET ON THE BIOCHEMICAL ANALYSIS OF RAT BLOOD (12 WEEKS OF EXPERIMENT)

Parameters	Control group	Experimental group
Glucose, mmol/L	4.95 (4.83–5.15)	7.55 (7.43–7.68)**
Cholesterol, mmol/L	1.6 (1.56–1.64)	2.25 (2.13–2.45)**
HDL, mmol/L	0.98 (0.97–0.99)	0.72 (0.69–0.74)**
Triglycerides, mmol/L	0.59 (0.48–0.69)	1.34 (1.28–1.4)**
Atherogenic index	0.64 (0.61–0.71)	2.17 (1.97–2.35)**

Note. ** – statistically significant difference between the experimental group and the control ($p \leq 0.01$).

TABLE 3

THE EFFECT OF A HIGH-CALORIE DIET ON CLINICAL AND BIOCHEMICAL URINALYSIS OF LABORATORY ANIMALS (12 WEEKS OF EXPERIMENT)

Parameters	Control group	Experimental group
Color	yellow	yellow
Volume, ml	2.1 (1.8–2.1)	2 (1.9–2.1)
Transparency	transparent	transparent
pH	7.4 (7.3–7.5)	7.4 (7.3–7.5)
Specific gravity, rel.units	1.05	1.07
Erythrocytes, u/ml	0	0
Leukocytes, u/ml	0	0
Glucose, mmol/L	0	0
Urobilinogen, mmol/L	0.2 (0.18–0.24)	0.22 (0.19–0.27)
Protein, g/L	0	0
Ketones, µmol/l	0	0
Creatinine, mmol/L	13.2 (8.4–17.7)	12.4 (8.8–16.9)

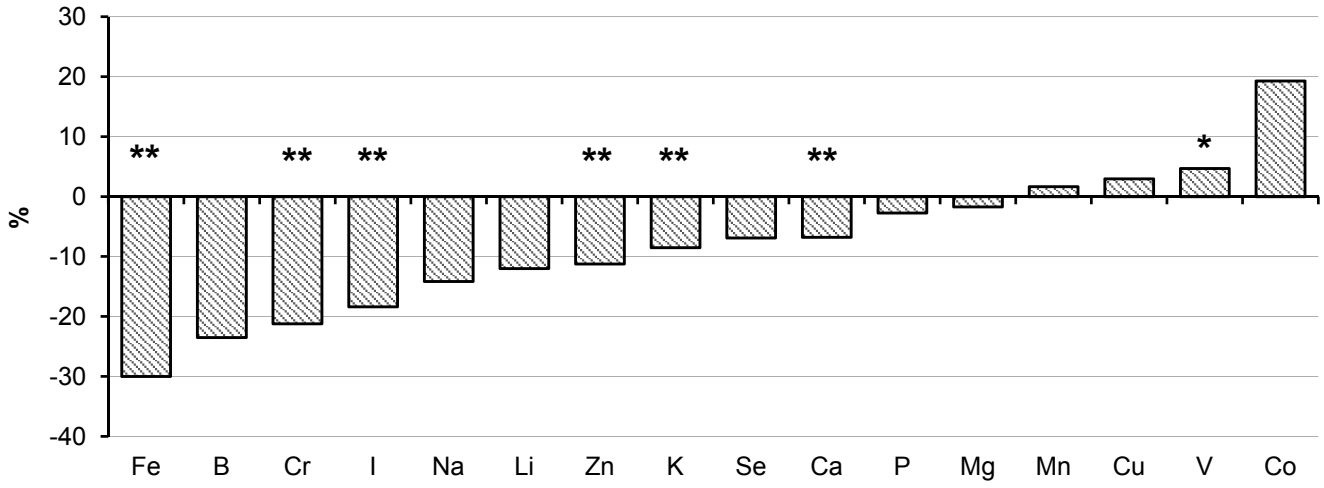

FIG. 3.

*Relative values of the content of chemical elements in the blood serum of the experimental group of rats (12 weeks of the experiment): X-axis (0) – the level of elements in the control group; * – statistically significant difference between the experimental group and the control (p ≤ 0.05);* ** **– statistically significant difference between the experimental group and the control (p ≤ 0.01)**

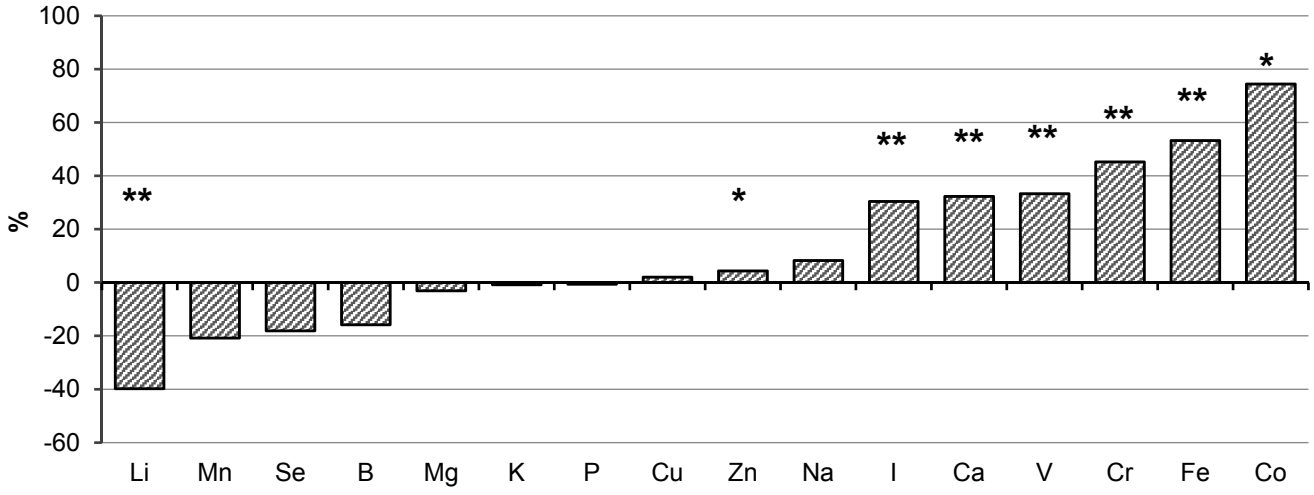

FIG. 4.

*Relative values of the content of chemical elements in the liver of the experimental group of rats (12 weeks of experiment): X-axis (0) – the level of elements in the control group; * – statistically significant difference between the experimental group and the control ($p \leq 0.05$); ** – statistically significant difference between the experimental group and the control ($p \leq 0.01$)*

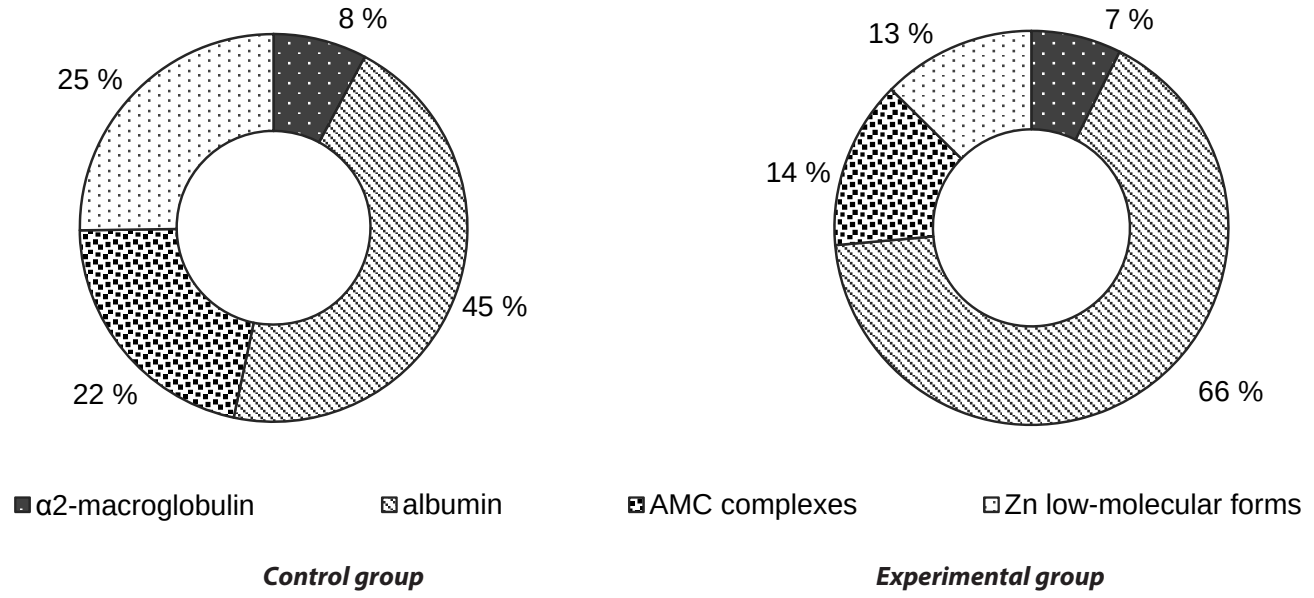

FIG. 5.

*Percentage distribution of zinc by fractions when exposed to a high-calorie diet (12 weeks of experiment): ** – statistically significant difference between the experimental group and the control ($p \leq 0.01$)*

forms of zinc. α2-macroglobulin level did not undergo significant changes throughout the study.

DISCUSSION

On average, the excess of the control body weight in animals of the experimental groups under the influence of the high-calorie diet by the end of the study was 44 %, which indicates the appearance of excess body weight. In the experimental group of animals, there was a statistically significant tendency for glucose, total cholesterol and triglyceride levels to increase while HDL levels decreased. The established impaired glucose tolerance as detected during the oral glucose tolerance test, as well as an increase in the atherogenic index, are predictors of diabetes and serve as risk factors for cardiovascular diseases [20]. The findings may indicate insulin resistance developing as a result of obesity, which in turn will aggravate the accumulation and retention of fat in the body. The re-

vealed disorders of carbohydrate and lipid metabolism are among the first typical signs of metabolic syndrome and may be associated with pro-inflammatory processes that may partially occur in adipose tissue [21].

There are a number of studies investigating the possibility of using the content of macro- and microelements in various biosubstrates as biomarkers of various disorders of carbohydrate and lipid metabolism, as well as their use as dietary supplements for metabolic correction [22]. The data obtained during the study show that exposure to a high-calorie diet for 12 weeks led to a statistically significant decrease in the levels of iron, chromium, iodine, zinc, potassium, calcium and an increase in vanadium in blood serum. In the liver tissues of the experimental group animals, a statistically significant decrease in lithium levels and an increase in zinc, calcium, vanadium, chromium, iron, and cobalt levels were noted. It is possible that such a pattern, associated with the reverse redistribution of certain chemical elements from the blood serum to the liver, is due to a defense mechanism. One study has demonstrated that interleukins released from activated phagocytic cells reduce the concentration of certain trace elements in the blood of experimental animals by redistributing them from the blood to the liver [23]. The identified chemical elements play an essential role in the regulation of carbohydrate and lipid metabolism [24]. Any changes in calcium concentration can interfere with the normal release of insulin, especially in response to glucose load, which in turn will affect body fat accumulation [25]. Chromium increases insulin sensitivity and is a component of glucose tolerance factor, and its decrease in blood serum in obese individuals with impaired glucose tolerance is recorded in many studies [26]. The inflammatory component of obesity, which leads to excessive hepcidin production, is believed to be one of the potential mechanisms of iron deficiency (hypoferremia) in obesity [27]. Variations in iodine, potassium, vanadium and cobalt concentrations in various disorders of carbohydrate and lipid metabolism are found in the works of several researchers, but require more detailed study.

Zinc cannot freely cross cell membranes, so there are a number of special carriers [28]. In this regard, the study of zinc complexation is important since various forms of this trace element are involved in a variety of biochemical and physiological processes, including Zn^{2+} transport into the liver and other organs; participation in the cytotoxicity and inflammation processes, deactivation of free radicals, etc. [29]. In this study, a decrease in total blood zinc level and an increase in liver tissue zinc level were detected, while a statistically significant percentage increase in the albumin fraction was recorded, with a decrease in AMC complexes and low-molecular-weight forms of zinc. Studies conducted by K.T. Smith et al. on isolated perfused rat intestines have shown that albumin is responsible for Zn^{2+} transport into the liver [30], which may explain the findings. The observed statistically significant changes in zinc distribution by individual forms in blood serum indicate a change in zinc metabolism due to high-caloric diet consumption. Evaluation of zinc fractions in blood serum revealed the presence of dysmetabolism of this metal when exposed to a high-calorie diet at a more subtle level. It is possible that these changes served as the molecular basis for changes in the total concentration of zinc in blood serum.

CONCLUSION

1. In the course of the study, it was found that the use of the developed high-calorie diet by Wistar rats for 12 weeks led to overweight in animals, impaired lipid metabolism and impaired glucose tolerance. These findings show that this diet makes it possible to simulate metabolic disorders and prove the effectiveness of its further use in studies of disorders of carbohydrate and lipid metabolism in laboratory animals.

2. In the course of the study, it was found that a high-calorie diet led to a statistically significant decrease in the levels of iron, chromium, iodine, zinc, potassium, calcium and an increase in vanadium in blood serum. In turn, there was a statistically significant decrease in the level of lithium and an increase in the level of zinc, calcium, vanadium, chromium, iron, and cobalt in the liver.

3. Due to the evaluation of the chemical forms of zinc, it was found that the detected decrease in zinc in blood serum and an increase in its concentration in the liver when using a high-calorie diet is associated with an increase in the serum albumin fraction of zinc affected by a decrease in AMC complexes and low-molecular forms of zinc.

The evidence suggests that a high-calorie diet leads to an imbalance of chemical elements, which may be one of the triggering mechanisms for the dysregulation of a number of physiological functions in living organisms. The changes revealed in metabolism may be a direct cause of the development of various functional disorders responsible for the onset of metabolic disorders and associated diseases.

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Conflict of interest

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

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GENETICS, PROTEOMICS AND METABOLOMICS

PROSPECTS FOR USING CRISPR-Cas9 SYSTEM IN THE TREATMENT OF HUMAN VIRAL DISEASES

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ABSTRACT

The aim. *To analyze the possibility of using the genetic mechanisms of CRISPR-Cas9 technology in the prevention and treatment of certain viral diseases.*
Materials and methods. *The search for publications was carried out in Russian and foreign literature using the following search engines: RSCI, Cyberleninka, eLibrary, PubMed, Cochrane Library, etc. A review of domestic and international scientific papers on the research topic was carried out using search keywords: CRISPR, genetic engineering, genome editing, Cas9, sgRNA.*
Results. *A review of using CRISPR-Cas9 method ("genetic scissors") as a gene therapy for some viral diseases was carried out, and its main advantages and disadvantages were revealed. An analysis of the data of scientific studies on genetic research methods over the past decade discovers the main aspects of CRISPR-Cas9 technology, modern classification and prospects for using this technology in clinical practice for the treatment and prevention of human viral diseases. The possibilities of creating a more versatile and stable version of the CRISPR-Cas9 technology are considered. Particular attention is paid to the technological difficulties and obstacles that scientists face when implementing this system for targeted use in clinical medicine.*
Conclusion. *One of the rapidly developing areas in science giving promising prospects for modern healthcare is genetic engineering, especially in cases where scientific developments are applied in clinical practice. The discovery of "genetic scissors" technology has revolutionized all medicine. Wide opportunities for developing new treatment methods for many viral diseases and creating conditions for their early prevention opened up for the medical community. In the future, with the introduction of this technology into clinical practice, it will become possible to treat diseases that have not previously responded to ongoing therapy and were considered incurable.*

Key words: *CRISPR, genetic engineering, genome editing, Cas9, sgRNA, viral diseases, infections*

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ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ СИСТЕМЫ CRISPR-Cas9 В ЛЕЧЕНИИ ВИРУСНЫХ ЗАБОЛЕВАНИЙ ЧЕЛОВЕКА

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РЕЗЮМЕ

Цель данной статьи *– провести анализ возможности применения генетических механизмов технологии CRISPR-Cas9 в профилактике и лечении некоторых вирусных заболеваний.*
Материалы и методы. *Поиск публикаций проводился в российской и зарубежной литературе в следующих поисковых системах: РИНЦ, Cyberleninka, eLibrary, PubMed, библиотека Cochrane и др. Проведён обзор отечественных и международных научных работ по теме исследования с использованием поисковых ключевых слов: CRISPR, генная инженерия, редактирование генома, Cas9, sgRNA.*
Результаты. *Проведён обзор использования метода CRISPR-Cas9 («генетических ножниц») в качестве генной терапии некоторых вирусных заболеваний, раскрыты его основные преимущества и недостатки. Анализ данных научных исследований в сфере генетических методов исследования за последнее десятилетие раскрывает основные аспекты технологии CRISPR-Cas9, современную классификацию и перспективы применения данной технологии в клинической практике с целью терапии и профилактики вирусных заболеваний человека. Рассматриваются возможности создания более многофункциональной и стабильной версии технологии CRISPR-Cas9. Особое внимание уделяется технологическим сложностям и препятствиям, которые встают перед учёными при внедрении данной системы для целевого применения в клинической медицине.*
Заключение. *Открытие «генетических ножниц» произвело революцию во всей медицине. Перед медицинским сообществом открылись широкие возможности для создания новых методов лечения множества вирусных заболеваний и создания условий для ранней профилактики. В перспективе при внедрении данной технологии в клиническую практику станет возможной терапия нозологий, ранее не отвечавших на проводимую терапию и считавшихся неизлечимыми.*

Ключевые слова: *CRISPR, генная инженерия, редактирование генома, Cas9, sgRNA, вирусные заболевания, инфекции*

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SECTION 1. INTRODUCTION

Viral infection poses a significant risk to human health, as evidenced by the significant morbidity and mortality from COVID-19 [1]. Viral infections are especially dangerous, the development of which occurs in a chronic form, characterized by periods of exacerbation and elimination of the virus, providing lifelong persistence of the virus. Currently existing antiviral drugs do not have high specificity and effectiveness, which makes it impossible to overcome biological barriers to eliminate the viral focus. At the same time, it is worthwhile noting the responsive adaptive ability of viruses to the methods of treatment used. Despite the improvement of the invasive properties of drugs to overcome biological barriers, the development of the disease has a long course, often leading to the death of the recipient, which is indicative for Retroviridae, Herpesviridae and Polyomaviridae families [2]. Despite the fact that different types of viruses have different ways of accessing the central nervous system (CNS), viral infections differ from other microorganisms by the fact that in the initial stage they persist on the periphery and thus gain access to the CNS structures. This occurs either by direct infection of nerve endings, or indirectly, by components of the circulatory system transporting the virus, overcoming the blood-brain barrier (BBB) in the CNS. These conditions require the development of more effective methods of treatment, which can be the technology of "genetic scissors" [3].

The development of modern technologies for human genome editing has made it possible (with the help of genetic engineering) to open up great prospects in the treatment of a number of diseases that were previously incurable. For the first time, CRISPR (clustered regularly interspaced short palindromic repeats) structures were discovered in the genome of *Escherichia coli* in 1987. Years later, it became known that these sequences can accumulate as a result of the invasion of mobile genome express (MGE): bacteriophages, plasmids and transposons – into the prokaryotic cell, which formed the basis of adaptive and inherited immunity in bacteria and archaea. Archaea are unicellular microorganisms that do not have a nucleus, as well as any membrane organelles, and represent the domain of living organisms.

The molecular mechanism of this process was revealed using the example of lactic acid bacteria *Streptococcus thermophilus* by K. Makarova et al. (2006); then the CRISPR-Cas system was divided into three types depending on the type of Cas proteins and the mechanism mediated by its effectors. CRISPR-Cas9 is currently used in genetic engineering and belongs to type II in the classification of CRISPR-Cas systems [4]. As a result of this technology discovery, the cost of genetic engineering has decreased by 99 %. It is difficult to say how revolutionary CRISPR is, but it has a huge potential that can forever change the diagnosis and therapy of numerous diseases of mankind. Emmanuelle Charpentier and Jennifer Doudna (2020) were awarded the Nobel Prize in Chemistry for the development of a genome editing method – CRISPR-Cas9 technology [2, 3].

Regularly arranged in groups of short palindromic repeats, loci (CRISPR) are direct repeats of short nucleotide chains that differ from each other by spacers – structural sections of the DNA of a foreign genome. A leader sequence containing a promotor is located in the chain of the host cell's own genome before a number of repeats of loci, after structures encoding Cas proteins. The promoter is a sequence of DNA nucleotides recognized by RNA polymerase as a starting point for the start of transcription. These structural elements determine the ability of bacteria to recognize foreign invaders by isolating sections of their genome, developing genetic memory, which further determines the ability to carry out an intracellular immune response that responds to the degradation of foreign nucleic acids in the event of its repenetration [5–7].

The immune response mechanisms of CRISPR-Cas are based on three successive stages: adaptation, expression and interference. The invasion of foreign DNA into the cell initiates the first stage of adaptation, at which the bank of genetic memory is replenished in order to adapt immunity. The reaction of Cas1-Cas2 proteins in most CRISPR-Cas systems is carried out in complex with a protospacer adjacent motif (PAM) and double-stranded DNA (dsDNA) of phages and plasmids, releasing a protospacer as a result of two double-stranded breaks (DSB) [8]. The complex of two Cas1 dimers performing a catalytic function and one Cas2 dimer performing a structural function acts like an integrase.

An important participant in spacer generation is the RecBCD multi-protein complex in the role of helicase and nuclease, which performs preferential sampling by splitting dsDNA, causing degradation of the foreign genome until reaching the hotspot (Chi sites), since the acquisition of MGE is characterized by the need to differentiate foreign and native genetic structures in order to avoid self-aggression [9]. The integration of the degraded dsDNA MGE fragment, acting similarly to the mechanism of viral integrases and transposases, forms a spacer of nucleic acids of foreign DNA in the massive CRISPR structure. The process of integrating the spacer into the CRISPR array occurs as a result of a number of reactions.

The integration host factor (IHF) performs a U-shaped deformation of the host DNA, recognizes the leader-repeat boundaries, the target site, 5'-phosphate of the proximal leader repeat, to which 3'OH protoprostans are attached by a nucleophilic attack mechanism. The next step is to merge the 3'OH of another protoprostane strand with the opposite end of the first repeat, resulting in a palindromic motif structure in the CRISPR repeat, serving as an anchor for the Cas1-Cas2 complex, and determining the position of the second integration site [10]. It is worth mentioning the acquisition of a primed spacer, characterized by enhanced absorption due to complementarity with previously integrated MGE, which serves as a counter-strategy against phage mutants. However, the main mechanism of this phenomenon has not been fully studied [7].

At the expression stage, Cas genes are expressed and an array of CRISPR repeats and spacers are transcribed into a long precursor of CRISPR RNA (pre-crRNA). Cas proteins and auxiliary factors transform pre-crRNA into a short mature crRNA capable of directing Cas to target sequences for their recognition and destruction. CRISPR turns are predominantly palindromes, resulting in the formation of pre-crRNA hairpins, which are then recognized by Cas. Pre-crRNA is cut into crRNA segments containing unique sequences complementary to the structure of the foreign target.

There are differences in the frequency of transcription of some spacers at the locus from others, which are explained by the presence of promoter elements in the repeat sequence. This phenomenon determines the strategic benefit in the speed of transcription of certain spacers that protect against retrospectively relevant invasive elements, providing fast and effective defense in stressful situations. The interference stage is a complex action of crRNA and Cas protein complexes that recognize and degrade the structures of foreign nucleic acids [5, 6, 11]. Each of the presented stages of intracellular immunological protection has its own characteristics depending on the type of CRISPR-Cas system [7].

SECTION 2. CHARACTERISTICS OF CRISPR-Cas CLASSES AND TYPES

All the diversity in the CRISPR-Cas system is usually differentiated into two classes: Class 1 – types I, III and IV; Class 2 – types II, V and VI [12].

Updated classification of CRISPR-Cas Class 1 systems

Class 1 (types I, III and IV) is represented by the crRNA multi-subunit effector complex. Type I is divided into six subtypes: I-A, I-B, I-C, I-D, I-E, I-F. Most type I pre-crRNA systems process ribonucleases (RNase) of the Cas6 family (Cas5d in subtype I-C). Cas6e remains bound to a repeating fragment of the 3′ end of the crRNA after processing. Subsequently, the cascade is assembled into a spiral-shaped seahorse. The crRNA is an integral part of the cascade connected along the backbone and bounded by the Cas5e at the 5′ end. The helical structure is represented by six bound Cas7e proteins, which adopt a conformation with protruding domains responsible for the reliable connection of subunits. Starting from the last nucleotide of the 5-inch sequence, the protruding subunit structures bend the crRNA at every sixth nucleotide.

Five nucleotides are lined up along the central domain to ensure efficient coupling of crRNA bases with the target DNA. Cas11e and Cas8e are defined as small and large cascade subunits, respectively. Recognition of PAM in a double-stranded DNA target is mediated by a large subunit that initiates the local cleavage of DNA chains and the binding of crRNA to the protospatial strand. The first eight PAM-proximal crRNA nucleotides are crucial in protospatial binding of the cascade complex, with the exception of the sixth, which does not bind to the target. As a result of binding of a non-target strand with two Cas11e subunits, the formation and stabilization of the R-loop structure occurs, accompanied by significant conformational changes in small and large subunits, which makes it possible to recruit Cas3 nuclease, causing structural changes in the protein that activate its ATP-dependent activity of the helicase.

As a result, Cas3 translocates and sequentially degrades the non-target DNA strand in the 3′-5′ direction, leaving a single-stranded DNA break (ssDNA) of 200–300 nucleotides (nt). However, these fragments may be intermediate degradation products, since the presented partial degradation of ssDNA can lead to the complete destruction of the foreign invader. It is believed that the complete degradation of the target DNA is mediated by host nucleases or by the powerful cascade-independent activity of ssDNA Cas3 nuclease, which has so far been observed only *in vitro* [7].

Today, it is interesting to study the fact that in the presence of an identical CRISPR-Cas structure and the mechanism of Cas3 participation, there are several distinctive features in the interference mechanism of type I subtypes. Subtypes I-A and I-E are the only systems containing a separate gene encoding a small subunit. In other subtypes, its genome is functionally replaced by Cas8. In addition, minimal cascade architecture is observed in subtype I-C, in which there is no Cas6 homologue and subtype I-Fv (subtype I-F variant), where large and small subunits are absent and functionally replaced by effector proteins Cas5fv and Cas7fv.

An interesting variation of the cascade overall shape was found in subtype I-F, in which the basis of the observation complex (Csy) has a short spiral step and almost forms a closed circle. Taken together, the results of numerous studies indicate significant genetic and functional plasticity in maintaining the overall architecture and module of RNA binding and processing (Cas6 and/or Cas5), backbone (Cas7), PAM recognition and R-loop stabilization [3].

Type III CRISPR-Cas systems use cascade-like complexes called Csm for III-A and Cmr for III-B, which structurally show high similarity to type I effector complexes. However, unlike the other interference mechanisms described, type III systems target both RNA and DNA substrates. The cleavage of DNA in a type III system depends on the transcription of the target sequence. Csm and Cmr assemble along a mature crRNA that is bound by Cas5 (Csm4/Cmr3) at the 5′ repeating end.

The basis of the complex is represented by proteins of the Cas7 family (Csm3 and Csm5 – for subtype III-A, Cmr4, Cmr6 and Cmr1 – for subtype III-B), while Cas11 (Csm2/Cmr5) and Cas10 are small and large subunits, respectively. Target cleavage is initiated by binding of a type III effector complex to the emerging target transcript dependent on the crRNA image. Cas7 (Csm3/Cmr4) subunits cleave single-stranded RNAs (ssRNA) at every sixth nucleotide. DNA division is carried out by the domain of the Cas10 subunit, which requires transcription of the target in both type III systems. RNAs belonging to the Csm6

or related Csx1 families are often associated with type III CRISPR-Cas systems.

Both Csm6 and Csx1 non-specifically destroy foreign transcripts and perform auxiliary functions during type III interference, even if they are not part of the effector complex [12]. The Cas10 subunit of the Csm complex, according to current data, not only mediates the cleavage of the target DNA, but also converts ATP into cyclic adenylates acting as secondary activators of Csm6 RNase. Cas10 production is associated with a complex of Csm and target RNA and is a regulatory mechanism that causes significant strategic interference in the development of a foreign invader [9].

CRISPR-Cas Class 2 system (types II, V and VI)

Type II systems use the Cas9 effector protein, a DNA double RNA endonuclease necessary for interference and immunity in type II systems. Differentiation in the A-, B- and C-subtypes of the type II system is based on the size of *Cas9* genes and the presence of type-specific genes. In addition to crRNA, Cas9 requires transactivating crRNA (tracrRNA), a small RNA that carries complementarity with repetitive crRNA regions. Once Cas9 has been linked to mature double-stranded RNA (tracrRNA:crRNA) or engineered single-stranded RNA (sgRNA) designed for genome engineering applications, Cas9 identifies the target DNA by recognizing PAM and the subsequent base pair of the guide RNA with DNA.

If the target shows sufficient complementarity with the guide RNA, Cas9 performs a double-stranded 3-nucleotide pair (bp) break proximal to the PAM. Cas9 is a leaf-like structure with a central locus containing a crRNA:DNA duplex. The α-helical recognition lobe (REC) and the nuclease lobe (NUC) are connected by an unordered linker and a highly conserved arginine-rich bridge helix forming several contacts with crRNA. The NUC lobe contains conservative nuclease domains HNH and RuvC and a variable C-terminal domain that interacts with PAM.

The study of molecular structures showed that the activity of Cas9 is regulated by the attachment of the guide RNA, which causes a change in the protein conformation towards the development of competence for binding to DNA and recognition of PAM. The surveillance complex associated with the guide RNA scans the DNA and, after recognizing complementary PAM structures in the non-target strand, induces DNA cleavage to allow the guide RNA to investigate the complementarity of the sequence from 10 to 12 nt in the PAM-proximal region of the target strand.

The pairing of bases between the guide RNA and the target DNA and additional conformational changes in Cas9 contribute to further invasion of the guide RNA beyond the domain sequence, thereby stabilizing the R-loop structure. Conformational activation of the HNH domain is associated with linker loops permutations between the HNH and RuvC domains. This allosteric bond between nuclease domains leads to a coordinated cleavage of the target strand by the HNH domain and the non-target strand by the RuvC domain [12, 13].

Type V CRISPR-Cas is divided into subtypes V-A, V-B and V-C, characterized by effector proteins Cas12a (formerly known as Cpf1), Cas12b (C2c1) and Cas12c (C2c3). Scientists have found out that phylogenetically these proteins originated independently from various transposon-associated nucleases of the TnpB family, which is manifested in the low similarity of the amino acid composition of these proteins with each other and with Cas9 [7].

Relative to Cas9 and Cas12b, the CRISPR-Cas subtype V-A effector protein Cas12a does not require tracrRNA for activation. After PAM recognition, a base pair between crRNA and target DNA is sufficient to activate effector proteins; as a consequence, Cas12a and Cas12b cleave both DNA strands, resulting in stepwise double-stranded breaks with 5- and 7-nt distal overhangs to PAM, respectively. Unlike Type II systems using different PAM structures located on a non-target strand, Cas12 proteins recognize the PAM of both DNA strands. It is important that Cas12b does not have a PAM recognition domain such as Cas9 or Cas12a. In addition, Cas12a and Cas12b require a duration and sequence of approximately 18 nt, which makes them promising alternatives to Cas9 in the matter of genome editing.

At the same time, in Cas12a, both DNA strands are cleaved in one catalytic site of the RuvC domain. Both DNA strands here are located in the same catalytic pocket of RuvC, so the target DNA strand is cleaved exactly by this domain. Details of the catalytic cleavage processes require further study; however, it is reliably known that Cas12a and Cas12b use similar mechanisms in the process of immunological protection of the host cell [9, 13].

Type VI

The data obtained in current studies made it possible to identify type VI systems, the structural feature of which is the content of two RxxxxH motifs. They are unique to the nucleotide-binding domains of higher eukaryotes (HEPN, higher eukaryotes and prokaryotes nucleotide-binding domain). The HEPN-containing effector protein Cas13, unlike other Class 2 effectors, is capable of cleaving ssRNA. Cas13 is activated by target ssRNAs, which is complemented by crRNA, resulting in degradation of not only target ssRNA, but also associated ssRNAs, similar to Csm6 and Csx1 enzymes in type III systems. However, tracrRNA is not required for Cas13 to function. Cas13a performs peripheral mismatch transfer in the crRNA:target ssRNA complex, but requires a central sequence of domains for RNase activity. Cas13a and Cas13b comprehensively treat repetitive regions of pre-crRNA, but biochemical and structural studies show different active sites for degradation of RNA-activated RNA and processing of pre-crRNA Cas13a.

When binding to pre-crRNA, Cas13a undergoes conformational changes that stabilize the crRNA and facilitate target binding. Binding of the target ssRNA increases the activity of RNase Cas13a, causing further conformational changes that morphologically bring the catalytic sites of HEPN domains into close proximity. Unlike the internal active sites of other Class 2 effectors, two Cas13a HEPN do-

mains form a composite active site on the outer surface of the enzyme [9, 12].

CRISPR-Cas9 is a promising genome editing technology, but there are a number of limitations and problems that need to be solved before its clinical use. One of the biggest obstacles is delivery efficiency, since CRISPR-Cas9 assumes an intracellular mechanism of action. CRISPR-Cas9 components can be delivered in various forms: in the form of small-sized mRNAs with convenient packaging or in the form of plasmid DNA encoding the Cas9 protein [8]. Other advantages of mRNA delivery are highly active gene editing and control over the volume of delivery into cells. Plasmids, on the other hand, have limitations in size and non-target effect, but they have the advantage of having stability and flexibility in design.

There are viral and non-viral methods of material delivery, such as electroporation, microinjection and lipid nanoparticles, but their therapeutic use is limited due to their relatively low delivery efficiency [8]. The most commonly used viral vectors for delivery in the therapeutic approach are adenoassociated virus (AAV), adenovirus and lentivirus due to their wide range of serotype specificity and relatively low immunogenicity [14]. The limitation of packaging capacity is a major problem for AAV-mediated CRISPR-Cas9 delivery. One solution to this limitation is to use a small version of Cas9 *Staphylococcus aureus* (SaCas9), which has the same gene editing efficiency as SpCas9, but a smaller size. Alternative is the use of dual AAVs to deliver Cas9-coding DNA and crRNA separately [4, 15].

Another problem that needs to be addressed is the potential non-targeted effect that causes pathogenic gene mutation and chromosomal translocations. In order to increase specificity, the CRISPR-Cas9 system was experimentally modulated by placing the *Cas9* gene under the control of a minimal HIV-1 promoter mediated by a trans-activator of transcription (Tat) to avoid overexpression of Cas9. In this case, Cas9 ribonucleoprotein Cas9/gRNA (Cas9 RNP) degrades after editing the target DNA, which leads to maximum target and minimum non-target effects. Nevertheless, it has been shown that the use of RNP in some types of cells can cause innate immune responses, leading to cytotoxicity in cells. Chemical synthesis and use of phosphatase crRNA to remove its 5′-ppp can inhibit innate immune responses and cytotoxicity [16].

Additionally, scientists faced the problem of the mechanism of non-homologous end joining (NHEJ), generation of resistant mutant viruses capable of counteracting Cas9/sgRNA by causing repair of DNA in the host cells. Various strategies have been proposed to prevent the development of viral resistance, such as the use of multiplex guide RNAs (gRNAs) to target multiple sites in the genome in order to reduce the generation of a viable escape mutant. The opposite alternative strategy is a combined approach of using CRISPR-Cas9 therapy with antiviral drugs and RNA interference molecules or short hairpin of RNA. In order to inhibit NHEJ DNA repair pathways, it is possible to use novel Cas9-like nucleases, such as Cpf1, which cleave the distal target site from PAM in order to reduce its binding to gRNA. Studies have shown that targeting a non-coding intergenic sequence is associated with viral interference activity, which significantly limits the creation of a viral escape-mutant [16].

SECTION 3. POSSIBILITIES OF USING CRISPR-CAS9 TECHNOLOGY IN INFECTIOUS DISEASES

The first experiments in the treatment of HIV-1/AIDS were carried out using CRISPR-Cas9 to suppress the expression of HIV-1 genes, focusing on long terminal repeats (LTR). The target sites were NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) binding clusters located in the U3 region of the LTR and R regions of TAR (trains acting responsive) sequences, which resulted in effective inhibition of HIV-1 virus genome transcription and replication. Another aspect is the possibility of CRISPR-Cas9 Cas9/gRNA virus genome cleavage in order to inactivate viral gene expression and limit viral replication in a latently infected T cell line, a promonocytic cell line and a microglial cell line with low genotoxicity [17–19].

There is evidence for mutational elimination of HIV-1 provirus by the effect of crRNA on the LTR sequence and the key viral replication genes and inactivation of the target site by mutation. Several studies have confirmed that CRISPR-Cas9 can degrade unintegrated HIV-1, as well as repair DNA mediated by NHEJ, which will lead to reduction in the integrated HIV-1 provirus [4, 20]. Circumcision of HIV-1 proviral DNA demonstrated the effectiveness of HIV-1 provirus disruption using AAV in combination with multiplex sgRNAs and SaCas9. The sgRNA/SaCas9 AAV-DJ/8 quadruplex injected intravenously into Tg26 mice can cleave HIV-1 proviral DNA and significantly reduce its replication. After intravenous injection of sgRNA/SaCas9 AAV-DJ/8 in bone marrow/liver and thymus (BLT) tissues infected with HIV-1, provirus cleavage was detected in the brain, colon, spleen, heart, and lungs. The first successful application of excision and elimination of HIV-1 SaCas9/gRNA proviral DNA *in vivo* supplied by AAV laid the foundation for the development of human clinical trials [21–23].

Inactivation of CCR5 and CXCR4 coreceptors using CRISPR-Cas9 technology made it possible to use CRISPR-Cas9 to block HIV-1 invasion by editing target receptors for HIV-1 – CD4 and CCR5 or CXCR4 coreceptors (CD4 impairment is not feasible). The use of this method has yielded the most promising results, since CCR5Δ32 transplantation has not been widely used. CRISPR-Cas9 in this case provides target sites with a simple design and plasmid construction. This method provides a huge potential in the diagnosis of CCR5 and CXCR4 expression disorders, which was proved in an experiment on Human Embryonic Kidney (HEK) 293T cells by transfection of Cas9 and sgRNA and induced pluripotent stem cells (iPSC). CCR5-modified iPSCs can usually differentiate into monocytes/macrophages resistant to HIV-1 infection [24].

To date, the preservation of the functional properties of CXCR4-deficient human T cells in a mouse model

has been proven, as well as the possibility of using recombinant CRISPR-Cas9 technology and piggyBac transport system to create mutant CXCR4 P191A with the function of inhibiting HIV-1 infection and without a deficiency of CXCR4 function, which makes it possible to edit CRISPR-Cas9 CXCR4 in mature post-thymic $CD4^+$ Human T cells for the purpose of HIV-1/AIDS therapy [25, 26].

An alternative strategy for reactivating host cell protection factors in HIV-1 infection is the activation of restriction factors, the expression of which is inhibited in HIV-1 infected cells. The effectiveness of two sgRNAs in Cas9-mediated induction of APOBEC3G (A3G) and APOBFC3B (A3B) restriction factor expression has been proven. However, research on the use of CRISPR-Cas9 technology to activate host cell factors to suppress HIV-1 infection is limited. At the same time, recently discovered restriction factors, such as serine incorporator five (SERINC5), human silencing hub (HUSH), consisting of TASOR, MPP8 and periphylin and capsid-binding factor for cyclic immune activation of GMP-AMP (cGAS) in macrophages and dendritic cells, NONO, may become new targets for consideration regarding this method use [26, 27].

Neurotropic human polyomavirus JC (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML) seen in patients with HIV-1/AIDS and patients undergoing immunomodulatory treatment of autoimmune disorders. Seroepidemiological studies have shown that JCV infection is widespread among 80 % of the world's population, due to constant asymptomatic persistence, and it manifests itself in immunodeficiency conditions. The JCV genome is represented by circular double-stranded DNA encoding sequences of an early viral T-antigen that determines virulence and ensures replication of new viral particles. The DNA of the virus is found only in the neuroglial cells of a healthy brain. The failure of currently used treatment options requires the development of an alternative approach. There is evidence of the use of CRISPR-Cas9 for inactivation of the genome region encoding the T-antigen by mutation of the Cas9- and gRNA-mediated N-terminal region of the T-antigen, leading to an impairment of its expression [28, 29].

One of the most common diseases is chronic **hepatitis B virus infection** (HBV), which is the main cause of liver cirrhosis and hepatocellular carcinoma. After infection, the viral genome is transported to the cell nucleus to be transformed into highly stable covalently closed circular DNA (cccDNA) [30, 31]. As a new approach to the treatment of chronic HBV, CRISPR-Cas9 can be used to inactivate cccDNA *in vitro* and *in vivo*. HBV genome mutations mediated by CRISPR-Cas9 lead to a significant decrease in the level of proteins HepG2, HepG2.2.15, HepG2-H1.3 and Huh-7. Current studies have shown the ability of the CRISPR-Cas9 system to eliminate the integrated cccDNA of the virus. The potential of antitumor use of CRISPR-Cas9 by targeted mutation of the *HBsAg* gene leading to suppression of tumor progression in hepatocellular carcinoma has also been demonstrated [32].

An important role in the study is played by the herpes virus family, which is also characterized by chronic primary persistence of infection and reactivation under certain physiological conditions. Modern nucleotide preparations only inhibit DNA polymerase, which is unpromising relative to the use of CRISPR-Cas9, which specifically breaks the viral genome [33].

Herpes simplex virus type 1 (HSV-1), having a dsDNA genome structure, is also a potential target for CRISPR-Cas9 in abrogation of epithelial and fibroblast cell infection. Also in the case of HSV-1, a viral mechanism of delaying early production of viral particles during latent persistence is strategically advantageous, since there is a possibility of the occurrence of escape insertions deletions (InDel). This technique requires further research, as there is a potential for the use of CRISPR-Cas9 using gRNA for the primary prevention of conditions such as viral-induced blindness associated with HSV-1, viral encephalitis and oral ulcers, Alzheimer disease, multiple sclerosis and epilepsy [34].

The Epstein – Barr virus (EBV) is a herpes γ-virus that has not received clinically approved therapy since its discovery in 1964 until today. Mediated by small interfering RNA (siRNA) disorders of the main *EBV* gene, *EBNA1*, an anti-EBV effect was achieved, but not the elimination of the virus genome from the host cell. Today, studies are being conducted on the T cells targeted effect on the EBV tumor antigen. However, in order to inactivate the genome of the herpes gamma virus from infected cells, a CRISPR-Cas9-based approach is applicable; the available experimental experience gives very encouraging results and is described in the reports of a number of researchers [33, 35].

Human cytomegalovirus (HCMV) is a dsDNA β-virus that, like other herpes viruses by the mechanism of viral delay, maintains the viral genome in infected cells without the production of virions. The results of experiments conducted on HCMV using the CRISPR-Cas9-gRNA multiplex approach to limit productive CMV infection in human cell lines led to the development of an escape mutant, but at the same time demonstrated successful inhibition of HCMV replication with the targeted effect of CRISPR-Cas9 on the key viral genes, such as *UL122/123* [33, 36].

Of particular interest are human papillomavirus, which are non-cell small dsDNA viruses. Infection occurs through skin or mucous epithelial cells, genital tissues and upper airways. Highly oncogenic HPV strains represent 95 % of the causes of anal cancer, 70 % of the causes of oropharyngeal cancer, 60 % of the causes of vaginal cancer, and are also the main cause of cervical cancer associated with high mortality [37].

Currently existing vaccines prevent infection with the virus, but do not provide protection for infected people, and are also significantly limited in the spectrum of HPV genotypes that do not have a cross-effect. The technique of using CRISPR-Cas9 in combination with the current available anticancer drug can be an effective treatment in oncology, especially in cases associated with HR-HPV. Experiments with intratumoral administration of CRISPR-Cas9 mediated by E6 and E7 HPV led to the development of inactivat-

ing InDel mutations, which is associated with the induction of p53 or pRb proteins, and also can lead to cell cycle arrest up to cell death. The use of CRISPR-Cas9 targeting the oncogenes E6 and E7 HPV16 in combination with Cisplatin *in vitro* and *in vivo* can be an effective therapy for cervical cancer in women [38].

CONCLUSION

Thus, the CRISPR-Cas9 system has a huge potential for use in human genetic engineering both *in vivo* and *in vitro*. However, there are certain issues related to the introduction of this technology into clinical practice. One of the major problems is the non-targeted system effect, which causes side mutation occurrence. A number of factors, such as the Cas9 expression level, target sequence, and quantification methods, determine the cleavage rate of the non-target Cas9 level.

It is likely that these mutations arise as a result of accidental DNA breakage and repair, but the mechanisms of repair, mutation and recombination of viral DNA in the host cell after CRISPR-Cas9 cleavage have yet to be studied. In some cases, non-target mutations may occur with a higher frequency than the necessary target sequential mutation, but the mechanism of this phenomenon remains unclear at this stage, so further advanced research in this area is needed.

In the future, research should focus on developing a new reliable and more targeted method to increase the specificity and focus of CRISPR-Cas9 technology. For the successful application of the CRISPR-Cas system in gene therapy, the new research strategy should also focus on improving the frequency and effectiveness of site-specific nuclease, especially in genome editing. The delivery of CRISPR-Cas9 components to target cells plays an important role in the successful application of this technology. The delivery system currently in use is not specific and highly efficient, especially in the field of biosafety, so safe and effective delivery methods will have to be developed.

Another problem of scientists is NHEJ, which leads to the generation of resistant mutant viruses capable of resisting Cas9/sgRNA, while causing DNA repair in host cells. Such limitations in the use of genome editing technology open up opportunities for new research; therefore, the use of CRISPR-Cas9 in clinical practice requires significant improvement in this method, the result of which may be the development of a promising direction for the treatment of a wide range of diseases.

Conflict of interest

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

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INFECTIOUS DISEASES

USING DOT-IMMUNOASSAY IN DECODING THE OUTBREAK OF PSEUDOTUBERCULOSIS IN THE TOMSK REGION

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ABSTRACT

Background. *Pseudotuberculosis remains a serious healthcare problem, which determines the expediency of developing the express methods for its early diagnosis. To detect the pathogen, we designed test system for dot-immunoassay (DIA) based on antibodies labeled with silver nanoparticles (SNPs) isolated from hyperimmune rabbit serum obtained against killed cells of Yersinia pseudotuberculosis of O:1b serovariant.*

The aim. *To assess the possibility of using dot-immunoassay for express identification of Y. pseudotuberculosis cultures isolated from clinical material and environmental objects at the initial stage of bacteriological study during laboratory diagnosis of the disease.*

Methods. *We used the materials from the outbreak of pseudotuberculosis in the Krylovskaya Boarding School of the Bakcharsky district of the Tomsk region in 2021. Specific antibodies from hyperimmune rabbit sera obtained against Y. pseudotuberculosis 3704 particulate antigen of O:1b serotype were labeled with SNPs and used in DIA on nitrocellulose membranes with visualization of reaction results with a solution of a physical developer. The presence of the causative agent of pseudotuberculosis in the test material was inferred by the formation of gray spots of different intensity (from 4+ to 1+).*

Results. *All Y. pseudotuberculosis strains isolated using bacteriological method on the second day of the study from clinical material obtained from sick people and environmental objects were detected in DIA at concentrations ≥ 3.1×10^4 microbial cells per milliliter (m.c./ml).*

Conclusion. *The designed test system for dot-immunoassay using SNPs as a marker of specific antibodies for the detection of Y. pseudotuberculosis in cultures isolated from swabs from vegetables and clinical material from patients, including those with mixed infection, allows us to detect a specific corpuscular antigen with a high sensitivity (≥ 3.1×10^4 m.c./ml), providing express identification of isolated cultures at the initial stage of bacteriological study.*

Key words: *Yersinia pseudotuberculosis, pseudotuberculosis, specific hyperimmune serum, colloidal silver nanoparticles, dot immunoassay*

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ИСПОЛЬЗОВАНИЕ ДОТ-ИММУНОАНАЛИЗА ПРИ РАСШИФРОВКЕ ВСПЫШКИ ПСЕВДОТУБЕРКУЛЁЗА В ТОМСКОЙ ОБЛАСТИ

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РЕЗЮМЕ

Актуальность. *Псевдотуберкулёз остаётся серьёзной проблемой здравоохранения, что определяет целесообразность разработки экспрессных методов его ранней диагностики. Для обнаружения патогена нами сконструирована тест-система для дот-иммуноанализа (ДИА) на основе меченых наночастицами серебра (НЧС) антител, изолированных из гипериммунной кроличьей сыворотки, полученной против убитых клеток Yersinia pseudotuberculosis серовариантa O:1b.*

Цель исследования. *Оценка возможности использования дот-иммуноанализа для экспресс-идентификации культур Y. pseudotuberculosis, выделенных из клинического материала и объектов окружающей среды, на начальном этапе бактериологического исследования при проведении лабораторной диагностики заболевания.*

Методы. *В работе использованы материалы по вспышке псевдотуберкулёза в МКОУ «Крыловская школа-интернат» Бакчарского района Томской области в 2021 г. Специфические антитела из кроличьих гипериммунных сывороток, полученных против корпускулярного антигена Y. pseudotuberculosis 3704 серотипа O:1b, метили НЧС и использовали в ДИА на нитроцеллюлозных мембранах. О наличии в исследуемом материале возбудителя псевдотуберкулёза судили по формированию пятен серого цвета разной интенсивности (от 4+ до 1+).*

Результаты. *Все исследованные штаммы Y. pseudotuberculosis, выделенные бактериологическим методом из клинического материала от больных людей и объектов окружающей среды, обнаруживались в ДИА в концентрациях ≥ 3,1 × 10^4 микробных клеток в 1 мл (м.к./мл).*

Заключение. *Разработанная тест-система для ДИА с использованием НЧС в качестве маркера специфических антител для обнаружения Y. pseudotuberculosis в культурах, выделенных из смывов с овощей и клинического материала от больных, в том числе с микст-инфекцией, позволяет с высокой чувствительностью (≥ 3,1 × 10^4 м.к./мл) обнаруживать возбудитель псевдотуберкулёза, обеспечивая экспрессную идентификацию изолированных культур на начальном этапе бактериологического исследования.*

Ключевые слова: *Yersinia pseudotuberculosis, псевдотуберкулёз, специфическая гипериммунная сыворотка, наночастицы коллоидного серебра, дот-иммуноанализ*

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The economic damage caused by the 35 most pressing human diseases of infectious origin for the Russian Federation in 2015 amounted to 549 billion rubles [1]. Pseudotuberculosis is a natural focal saprozoonosis with a fecal–oral transmission [2], which is realized through infected food consumed raw or insufficiently heat-treated [3–6]. As a psychrophilic microorganism, pseudotuberculous microbe is able to proliferate at low temperature [7], form biofilm on refrigeration equipment and food products, accumulate on raw vegetables during their storage from autumn to winter [8], which causes a potential biological hazard to humans [9].

Enteropathogenic yersinia are the third most common etiological factor in the European Union after salmonellosis and campylobacteriosis causative agents [10]; pseudotuberculosis is registered almost everywhere in Russia in the form of sporadic and outbreak morbidity [11]. The occurrence of mass epidemic manifestations of this infection is also possible in natural and man-made emergency situations due to the occurrence of epizootics among rodents and, as a consequence, contamination of water and food by a causative agent in the areas of the affected population [12].

The effectiveness of anti-epidemic measures carried out in foci is determined by the timely detection of an etiological agent, the expressiveness and reliability of laboratory diagnostics. The most promising methods are those aimed at detecting the causative agent in biological material, food, water, swabs from environmental objects [13]. Dot-immunoassay (DIA) with antibodies labeled with silver nanoparticles (SNPs) is characterized by high sensitivity, compactability of analytical system (sample volume ~1–2 μl), ease of implementation and quick results (~1.5–2 hours), the possibility of non-instrumental use in field conditions. The test systems designed by us for DIA based on specific antibodies labeled with SNPs have been successfully tested for the detection of causative agents of plague, brucellosis, tularemia and botulinum toxin [14, 15].

THE AIM OF THE STUDY

To assess the possibility of using dot-immunoassay for express identification of *Y. pseudotuberculosis* cultures isolated from clinical material and environmental objects at the initial stage of bacteriological study during laboratory diagnosis of the disease.

MATERIALS AND METHODS

Experimental researches were conducted in accordance with the decision of the Council of the Eurasian Economic Commission No. 79 dated November 3, 2016 "On approval of the Rules of Good Clinical Practice of the Eurasian Economic Union", Order of the Ministry of Health of the Russian Federation No. 199n dated April 1, 2016 "On approval of the Rules of Good Laboratory Practice".

When working with animals, we were guided by: GOST 34088-2017 "Guidelines for the accommodation and care of laboratory animals. Rules for keeping and care of farm animals" (applicable from August 1, 2018); Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the Protection of Animals used for Scientific Purposes; International Guiding Principles (Code of Ethics) for Biomedical Research Involving Animals (CIOMS, Geneva, 1985); European Convention for the Protection of Vertebrates Animals used for Experimental and other Scientific Purposes (Strasbourg, March 03, 1986).

We used materials on the etiological interpretation of the outbreak of pseudotuberculosis (from January 25 to February 2, 2021) at the Krylovskaya Secondary Boarding School for students with disabilities in the Bakcharsky district of the Tomsk region. 34 of affected people are reported to have the final diagnosis of "Pseudotuberculous infection". Two children had mixed infection of pseudotuberculosis + rotavirus infection; one child had mixed infection of pseudoturbeculosis + associated viral infection (rotavirus + norovirus infection). Secondary diagnosis: one child is a rotavirus carrier; 2 children are enterovirus carriers.

All patients were examined for causative agents of relevant intestinal infections by bacteriological method, polymerase chain reaction (PCR) (AmpliSens® OKI screen-FL, "AmpliSens *Yersinia enterocolitica/pseudotuberculosis*-FL", AmpliSens® Enterovirus-FL). The research was carried out by specialists of the laboratories of the Center for Hygiene and Epidemiology in the Tomsk Region and the bacteriological laboratory of the Tomsk Regional Clinical Hospital.

According to the results of the study, specific fragments of *Y. pseudotuberculosis* DNA were found in 7 samples by PCR. Rotavirus RNA was detected in two samples, norovirus RNA was detected in one sample and enterovirus RNA was detected in two samples. *Y. pseudotuberculosis* was isolated by bacteriological method in 6 cases from stool samples and in 1 case from urine sample.

27 samples of coprofiltrates from patients (material in a peptone-potassium medium) were sent to the Center for Surveillance of Yersinioses of Saint Petersburg Pasteur Institute of Epidemiology and Microbiology. Samples after "cold enrichment" were examined by PCR with AmpliSens® *Yersinia enterocolitica/pseudotuberculosis*-FL hybridization-fluorescence detection (Central Research Institute of Epidemiology, Moscow) with a set of reagents for detecting and differentiating DNA of virulent and avirulent strains of *Yersinia enterocolitica* and strains of *Yersinia pseudotuberculosis* in objects environment and clinical material. As a result, a culture of a pseudotuberculous microbe was isolated by bacteriological method in all samples where *Y. pseudotuberculosis* DNA was detected.

Specific hyperimmune serums were obtained by immunizing Chinchilla rabbits with *Y. pseudotuberculosis* inactivated strain No. 3704 of O:1 serotype [15]. Immunoglobulins G (IgG) isolated from the obtained sera [16] were labeled with SNPs of 5–9 nm size [17] and used in DIA,

which was carried out in a traditional way [14], involving the adsorption of the investigated material on the nitrocellulose membrane (NCM), blocking free areas of the solid phase with an inert protein solution. The detection of antigens adsorbed on NCM was carried out using labeled IgG, followed by visualization of the reaction results with a photodeveloper consisting of methol, citric acid and silver nitrate [18]. The presence of pseudotuberculosis causative agent in the studied material was judged by the formation of gray spots of different intensity (from 4+ to 1+) in the sample application sites, depending on the dilution of the studied material used.

RESULTS AND DISCUSSION

The work presents the results of a study of 8 *Y. pseudotuberculosis* cultures isolated from patients and 3 cultures isolated from swabs from vegetables during an epidemic outbreak in the Tomsk region, received by the Center for Indication and Laboratory Diagnostics of Irkutsk Antiplague Research Institute of Siberia and Far East for final identification. Considering that the bacteriological study is usually carried out for 15 (material from patients) and 21 days (material from environmental objects) with periodic seeding on the 2nd or 3rd, 5th, 7th, 10th or 15th and 21st days [19], we used dot-immunoassay as the method of express identification in the study of isolated cultures at the initial stage of bacteriological research. Culture seeding was performed in bromothymol blue medium (State Research Center for Applied Biotechnology and Microbiology, Obolensk) and incubated in a thermostat at +28 °C for 48 hours. At the stage of primary identification from the selected suspicious colonies (2–3 days), a suspension with a concentration of ~10^7 microbial cells in 1 ml (mc/ml) was prepared for analysis in DIA, inactivated by boiling in a water bath for 20 minutes, and after monitoring the specific sterility, each sample was titrated to determine the minimum concentration of the pathogen detected in dot-immunoassay, and each dilution of the material was applied to the membrane in a volume of 1 µl.

Detection of *Y. pseudotuberculosis* strains adsorbed on NCM in DIA (No. 1-11, No. 12 – *Y. pseudotuberculosis* 3704 O:1) was carried out at different concentrations. The analysis time did not exceed two hours. Samples containing pseudotuberculous microbe were detected on NCM in the form of gray spots with an intensity from 4+ to 1+ at concentrations of 12.5×10^4 – 3.1×10^4 mc/ml (Fig. 1).

The studied samples were determined in DIA in the following concentrations: strain No. 1 – 12.5×10^4 mc/ml; strain No. 2 – 12.5×10^4 mc/ml; strain No. 3 – 3.1×10^4 mc/ml; strain No. 4 – 3.1×10^4 mc/ml; strain No. 5 – 12.5×10^4 mc/ml; strain No. 6 – 6.2×10^4 mc/ml; strain No. 7 – 6.2×10^4 mc/ml; strain No. 8 – 3.1×10^4 mc/ml; strain No. 9 – 3.1×10^4 mc/ml; strain No. 10 – 6.2×10^4 mc/ml; strain No. 11 – 3.1×10^4 mc/ml. The control strain *Y. pseudotuberculosis* 3704 O:1 from the collection of the Department of Epidemiology, Irkutsk Antiplague Research Institute of Siberia and Far East, was detected at a concentration of 3.1×10^4 mc/ml. As negative controls, a sample of swab from a cabbage from a supermarket and a titration buffer were examined. All positive results obtained in dot-immunoassay were fully correlated with PCR and confirmed by further isolation of *Y. pseudotuberculosis* by a bacteriological method carried out according to the standard analysis scheme.

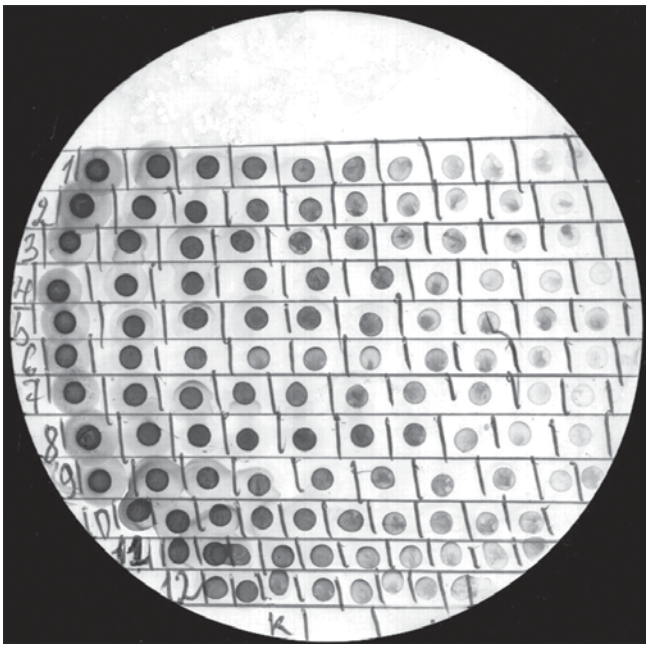

FIG. 1.
Detection of the causative agent of pseudotuberculosis in infectious material in dot-immunoassay: horizontal rows 1–8 – titrations of the studied corpuscular antigens from sick people; horizontal rows 9–11 – titrations of the studied corpuscular antigens from vegetables wipe samples; horizontal row 12 – Y. pseudotuberculosis 3704 O:1; "к" – negative controls

CONCLUSION

Thus, the reliably established possibility of using dot-immunoassay as an express method for determining *Y. pseudotuberculosis* in biological material and environmental objects at the stage of primary identification of selected suspicious colonies (2–3 days) during bacteriological analysis is of practical interest when conducting laboratory diagnostics of the disease in a shorter period of time and, consequently, accelerated verification diagnosis of pseudotuberculosis occurring with a variety of symptoms and syndromes, as well as during microbiological monitoring in order to control the effectiveness of anti-epidemic measures, including in the event of biological threats.

The results obtained indicate the high sensitivity of the developed test system for detecting the causative agent of pseudotuberculosis in DIA in the minimum volume of the studied sample (1 µl) for two hours.

The presented data demonstrate the high efficiency of DIA in express identification of the causative agent of pseudotuberculosis at the first stages of isolation of cultures from native material, which is confirmed by the results of a parallel study of the received samples in PCR and further isolation of *Y. pseudotuberculosis* by a bacteriological method carried out according to the standard analysis scheme.

Conflict of interest

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

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X-RAY DIAGNOSTICS OF TUBERCULOSIS IN THE SCREENING OF PATIENTS WITH HIV INFECTION

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ABSTRACT

Background. *The main method of screening of patients with HIV infection to detect pulmonary tuberculosis is X-ray diagnostics. A comparative assessment of changes in lung tissue in different methods of X-ray diagnostics and at different levels of $CD4^+$ cells seems relevant.*

The aim of the study. *To compare plain radiography and computed tomography of the lungs as screening methods for detecting tuberculosis in patients with HIV infection at various stages of immunosuppression.*

Material and methods. *396 patients with HIV registered at the AIDS Center were examined using plain lung radiography (PLR) and computed tomography (CT). To search for mycobacterium tuberculosis, we used bacterioscopy of a smear with Ziehl – Neelsen staining; HAIN-GenoType MTBDRplus technique; inoculation of BACTEC MGIT 960 liquid media; inoculation of Löwenstein – Jensen dense medium. Statistical processing of numerical material was carried out using the Statistica 5.5 program with a significance level $p \leq 0.05$; Pearson χ^2 criterion was used for the analysis of qualitative features.*

Results. *When comparing the results of PLR and CT, the differences were found. When using PLR, the focal lung lesions were described more often ($\chi^2 = 40.79$; $p = 0.00001$), according to CT data, they turned out to be fibrosis ($\chi^2 = 2.33$; $p = 0.1269$). When comparing the PLR and CT data, the differences were obtained in the reporting of pulmonary fibrosis ($\chi^2 = 20.78$; $p = 0.00001$), focal lung lesions ($\chi^2 = 40.79$; $p = 0.00001$), dissemination ($\chi^2 = 9.16$; $p=0.0025$).*

Conclusion. *When screening HIV-infected patients (at the standard of using plain radiography twice a year) it should be taken into account that CT provides more precise differentiation of focal lung lesions and pulmonary fibrosis, earlier detection of dissemination syndrome and ground-glass, especially at severe immunodeficiency with a decrease in $CD4^+$ T lymphocytes down to 200 cells and less. Timely appointment of CT study will improve the effectiveness of tuberculosis detection at the stage of screening in AIDS centers.*

Key words: *plain radiography, computed tomography, tuberculosis, HIV infection*

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ЛУЧЕВАЯ ДИАГНОСТИКА ТУБЕРКУЛЁЗА В СКРИНИНГЕ ПАЦИЕНТОВ С ВИЧ-ИНФЕКЦИЕЙ

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РЕЗЮМЕ

Обоснование. *Основным методом скрининга ВИЧ-инфицированных пациентов для выявления туберкулёза лёгких является лучевая диагностика. Сравнительная оценка изменений в лёгочной ткани при различных методах лучевой диагностики и различном уровне $CD4^+$-клеток представляется актуальной.*

Цель исследования. *Сравнить обзорную рентгенографию и компьютерную томографию лёгких как методы скрининга по выявлению туберкулёза у пациентов с ВИЧ-инфекцией с различными стадиями иммуносупрессии.*

Материал и методы. *Методами обзорной рентгенография лёгких (ОРГ) и компьютерной томографии (КТ) обследованы 396 пациентов с ВИЧ, состоящих на учёте в СПИД-центре. Для поиска микобактерий туберкулёза применялись бактериоскопия мазка с окраской по Цилю – Нильсену; методика по HAIN-GenoType MTBDRPlus; посев на жидких средах BACTEC™ MGIT™ 960; посев на плотных средах Левенштейна – Йенсена. Статистическую обработку числового материала проводили с использованием программы Statistica 5.5 с уровнем значимости $p \leq 0,05$; для анализа качественных признаков использовали критерий χ^2 (Пирсона).*

Результаты. *При сопоставлении результатов ОРГ и КТ отмечены различия. При ОРГ чаще описывались очаговые тени ($\chi^2 = 40,79$; $p = 0,00001$), которые по данным КТ оказались фиброзом ($\chi^2 = 2,33$; $p = 0,1269$). При сравнении данных ОРГ и КТ получены отличия в описании фиброза лёгочной ткани ($\chi^2 = 20,78$; $p = 0,00001$), очаговых теней ($\chi^2 = 40,79$; $p = 0,00001$), диссеминации ($\chi^2 = 9,16$; $p = 0,0025$).*

Заключение. *При проведении скрининга ВИЧ-инфицированных пациентов (при стандарте применения обзорной рентгенографии 2 раза в год) необходимо учитывать, что КТ позволяет чётче дифференцировать очаговые тени и фиброз лёгочной ткани, раньше выявлять синдром диссеминации и «матовое стекло», особенно при выраженном иммунодефиците при снижении $CD4^+$ Т-лимфоцитов менее 200 клеток. Своевременное назначение КТ позволит повысить эффективность выявления туберкулёза на этапе скрининга в центрах СПИД.*

Ключевые слова: *обзорная рентгенография, компьютерная томография, туберкулёз, ВИЧ-инфекция*

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OBJECTIVES

The HIV infection epidemic situation in Russia, with an emerging trend towards improvement, still remains dramatic [1–2]. Significant achievements in reducing population morbidity and mortality from tuberculosis (TB) with a high prevalence of HIV infection in Russia reduce the stabilization rate of the tuberculosis situation [3]. HIV infection has become the most significant risk factor for activation of latent tuberculosis infection caused by *M. tuberculosis* [4]. This situation is largely proven by the spread of tuberculosis among HIV-infected people (TB/HIV). During the period from 2005 to 2018, the incidence of TB/HIV in Russia increased 4 times – from 2.1 to 8.5 per 100 thousand population – and was accompanied by a significant increase in the number of deaths, which was due to the late detection of HIV infection, when the course of the disease acquired a severe and sometimes irreversible character [5].

Currently, the majority of patients with HIV infection are observed in the AIDS centers, where check-ups allow for the timely tuberculosis detection. In general healthcare centers, when dealing with symptoms of lung disease, both tuberculosis and HIV infection can be detected for the first time [6, 7].

A method of tuberculosis screening in patients with HIV infection is X-ray diagnostics, carried out twice as often as in the general population (twice a year). Radiologic changes are still the most informative, since they allow detecting local forms of tuberculosis in varying degrees of severity and localization in 100 % of cases when examining the lungs. Increasingly, in AIDS centers, computed tomography (CT) is currently being used as screening, which makes it possible to increase the informative value of the study. The revealed during X-ray diagnostics changes are not specific and may be characteristic of other lung diseases. If changes are detected on the X-ray and/or CT, a diagnostic minimum examination for tuberculosis is performed. It is important to prove the specificity of the process by detecting *Mycobacterium tuberculosis* (MBT) [7–10].

Clinical and radiological manifestations of the tuberculosis process in patients with HIV infection depend on the degree of immunosuppression. At advanced stages of immunodeficiency, the nature of inflammation changes, and generalization of the process is possible [11–13].

Tuberculosis combined with HIV infection is characterized by atypical manifestations, including X-ray ones, which complicates diagnosis [14, 15]. In severe immunodeficiency (CD4 < 100 cells/μL), clinical symptoms are 4–8 weeks ahead of the appearance of dissemination; in most patients, changes characteristic of tuberculosis cannot be detected on the X-ray [3].

During the initial exam of X-ray images, the radiologist suspects tuberculosis in 34.5 % of cases; at the same time, characteristic changes for the early process stages are noted only in 20.7 % of patients [5, 16].

The features of HIV-associated tuberculosis are constantly being studied by both Russian and foreign authors. There are contradictions about the frequency of bacterial excretion in such patients; most authors note difficulties in verifying the diagnosis [17]. Determining the role of X-ray diagnostics among the methods of detecting tuberculosis in HIV-positive individuals is considered as an important aspect [18].

THE AIM OF THE STUDY

To compare plain radiography and computed tomography as screening methods for detecting tuberculosis in HIV infected-patients with various stages of immunosuppression.

MATERIALS AND METHODS

Study design. A retrospective non-randomized study was performed within 2019–2020. The subjects of the study were 396 patients of both genders meeting the inclusion criteria, aged 25 to 65 years (average age – 40.95 ± 8.02 years).

Compliance criteria. The inclusion criteria for this study were: age 18 and older, diagnosis of HIV infection, and follow-up care at the AIDS center.

Procedure situation. The study included patients undergoing follow-up care at the Samara Regional Center for the Prevention and Control of AIDS and Infectious Diseases.

Recording outcomes methods. The study included patients consulted by a phthisiatrician at the AIDS Center. A standardized questionnaire including data on HIV infection and tuberculosis infection was filled out by all patients (by continuous sampling method), and the results of annual plain lung radiography (if available) were taken into account. Before the study, all patients underwent plain lung radiography (PLR) in two anatomical projections and computed tomography. MBT was searched in sputum and/or bronchoalveolar fluid (in case of bronchoscopy) of the patient. To detect MBT in all patients, sputum smear bacterioscopy techniques (BALG, bronchoalveolar lavage) with Ziehl – Neelsen staining, a molecular genetic method based on hybridization technology (HAIN-GenoType MTBDRplus), liquid media inoculation in the automated BACTEC™ MGIT™ 960 system, inoculation of Löwenstein – Jensen dense medium were used. Data for the analysis were entered into an MS Excel spreadsheet (Microsoft Corp., USA) and did not contain any personal data of the study subjects [19].

Ethical review. Written informed consent was obtained from each study participant before performing the procedures. The research protocol was approved by the Bioethics Committee of the Samara State Medical University of the Ministry of Health of the Russian Federation (Protocol No. 211 dated October 7, 2020).

Statistical analysis. Statistical data processing was carried out using Statistica 10 software package (StatSoft Inc., USA). Normality verification of the quantitative parameters distribution was carried out using the Kolmogorov – Smirnov test. For quantitative parameters in the compared groups, the arithmetic mean and root-mean-square (standard) errors of the mean (M ± m) were estimated,

and confidence interval limits (CI) were calculated. Qualitative features were analyzed by examining their frequencies through contingency tables using a chi-squared test χ^2 (Pearson's test). The critical value of the statistical significance level when testing the null hypotheses was taken to be 0.05. If the achieved level was exceeded, the null hypothesis was accepted.

RESULTS

A survey of 396 HIV-infected patients who had been registered at the AIDS Center for at least a year was conducted. A quarter of them (24.24 %) were with severe immunosuppression – the number of $CD4^+$ cells < 50 cells/μL (Table 1).

TABLE 1

DISTRIBUTION OF PATIENTS WITH HIV INFECTION BY LEVEL OF $CD4^+$ T LYMPHOCYTES

Level of $CD4^+$ T-cells, cells/μL	Total	
	abs.	%
< 50	96	24.24
50–99	53	13.38
100–199	75	18.94
200–349	76	19.19
350–499	28	7.07
> 500	68	17.17

The phthisiatrician consulted 396 patients. Male were the majority – 263 (66.4 %); female – 133 (33.6 %); average age – 40.57 ± 0.39 years (95% CI: 39.80–41.34). 250 (63.1 %) were unemployed of working age, 52 subjects had a profession and worked in their own specialist areas (13.1 %); the rest had casual employment. The most frequent route of HIV infection is through the injecting drugs use (IDU) – 57.6 % of all cases ($\chi^2 = 18.18$; $p = 0.00001$).

The limitation of HIV infection detection within three years was registered in 55 % of all cases ($\chi^2 = 8.08$; $p = 0.0045$); in other patients — more than three years. The majority of patients (75 %) were registered in the last 3 years ($\chi^2 = 70.32$; $p = 0.00001$).

When comparing the results of the plain lung radiography with the computed tomography data, differences in the reporting were noted. Focal lung lesions ($\chi^2 = 40.79$; $p = 0.00001$) were more often described during the plain lung radiography, which, according to CT data, in some cases were assessed as limited fibrosis ($\chi^2 = 2.33$; $p = 0.1269$). The preliminary diagnosis of focal tuberculosis with the reporting of a focal lung lesion on the X-ray was cancelled after CT examination in 12 patients. According to the CT, some changes interpreted as focal lung lesions were assessed as normal or fibrosis. As per the CT, the dissemination syndrome was detected much more often ($\chi^2 = 9.16$; $p = 0.00.259$) (Table 2). When comparing X-ray and CT data, statistically significant differences were obtained in the reporting of pulmonary fibrosis ($\chi^2 = 20.78$; $p = 0.00001$), focal lung lesions ($\chi^2 = 40.79$; $p = 0.00001$), dissemination ($\chi^2 = 9.16$; $p = 0.0025$ (Pearson's test)).

Patients examined for tuberculosis were classified depending on changes in lung tissue according to CT scans of the chest organs (chest CT) and CD4 cell levels (Table 3). Lung tissue dissemination was observed in 39 % of cases in patients with level of CD4 cells < 50 cells/μL; in half of the cases, changes in the X-ray were not detected due to low contrast. When reported on chest CT, these changes were interpreted as "ground-glass", which is a "signal" of the dissemination syndrome. As the level of CD4 cells increased, lung tissue dissemination was less common. Lung tissue changes in the form of infiltration focus were found in 30 % of cases ($n = 43$) in patients with CD4 cells < 50 cells/μL level. At follow-up diagnosis, 72.1 % of patients from this group ($n = 31$) were diagnosed with pulmonary tuberculosis. The majority of patients (73.5 %; $n = 50$) from the group with CD4 cells > 500 cells/μL

TABLE 2

RESULTS OF PLAIN RADIOGRAPHY AND COMPUTED TOMOGRAPHY

Changes in lung tissue	PLR	CT	Pearson's test (χ^2; p)
Focal lung lesion	91 (22.98 %)	27 (6.82 %)	$\chi^2 = 40.79$; $p = 0.00001$
Limited dimming (focus)	167 (42.17 %)	146 (36.87 %)	$\chi^2 = 2.33$; $p = 0.1269$
Lung tissue dissemination	22 (5.56 %)	51 (12.88 %)	$\chi^2 = 9.16$; $p = 0.0025$
Rounded lesion in the pulmonary field	3 (0.76 %)	3 (0.76 %)	$\chi^2 = 0.00$*; $p = 1.0000$
Pleural effusion	6 (1.52 %)	6 (1.52 %)	$\chi^2 = 0.00$*; $p = 1.0000$
Limited fibrosis	37 (9.34 %)	83 (20.96 %)	$\chi^2 = 20.78$*; $p = 0.00001$
Norm (without pathology)	70 (17.68 %)	80 (20.20 %)	$\chi^2 = 0.82$*; $p = 0.3645$
Total	396	396	

Note. * – Pearson's chi-square test with Yates' correction for continuity.

level were not diagnosed with pulmonary tuberculosis upon further examination.

According to the results of a complete physical examination by a phthisiatrician, 174 patients were diagnosed with tuberculosis. Among the active forms of pulmonary tuberculosis, infiltrative – 63.8% of cases ($\chi^2 = 17.66$; $p = 0.00001$) and disseminated – 26.4% of cases ($\chi^2 = 58.00$; $p = 0.00001$) were more common (Table 4).

TABLE 3

DISTRIBUTION OF CHANGES IN LUNG TISSUE ON CHEST CT BY THE LEVEL OF CD4 CELLS IN PATIENTS EXAMINED FOR TUBERCULOSIS

Changes in lung tissue according to chest CT	Level of CD4⁺ T-cells, cells/μL					
	< 50	50–99	100–199	200–349	350–499	> 500
Focal lung lesion (*n* = 27)	7 (26.0 %)	2 (7.4 %)	4 (14.8 %)	10 (37.0 %)	–	4 (14.8 %)
Limited dimming (focus) (*n* = 146)	43 (29.0 %)	20 (13.7 %)	38 (26.0 %)	15 (10.8 %)	10 (6.8 %)	20 (13.7 %)
Lung tissue dissemination (*n* = 51)	20 (39.2 %)	13 (25.5 %)	7 (13.7 %)	10 (19.6 %)	1 (2.0 %)	–
Rounded lesion in the pulmonary field (*n* = 3)	–	–	1 (33.3 %)	–	–	2 (66.7 %)
Pleural effusion (*n* = 6)	–	3 (50.0 %)	1 (16.7 %)	1 (16.7 %)	1 (16.7 %)	–
Limited fibrosis (*n* = 83)	12 (14.5 %)	5 (6.0 %)	12 (14.5 %)	23 (27.7 %)	9 (10.8 %)	22 (26.5 %)
Without pathology (*n* = 80)	14 (17.5 %)	10 (12.5 %)	12 (15.0 %)	17 (21.3 %)	7 (8.7 %)	20 (25.0 %)
Total (*n* = 396)	96 (24.2 %)	53 (13.4 %)	75 (18.9 %)	76 (19.2 %)	28 (7.1 %)	68 (17.2 %)

TABLE 4

CLINICORADIOLOGIC DIAGNOSES

Total	Diagnosis	abs. (*n* = 396)	%
Active pulmonary tuberculosis (*n* = 174)	Disseminated tuberculosis	46	26.44
	Infiltrative tuberculosis	111	63.79
	Focal tuberculosis	17	9.77
No evidence of pulmonary tuberculosis (*n* = 222)	Residual changes of tuberculosis (calcifications, pneumofibrosis)	13	5.86
	Community-acquired pneumonia	53	23.87
	Pleurisy	6	2.70
	Fibrosis	70	31.53
	Without pathology	80	36.04

TABLE 5

DISTRIBUTION OF LUNG TISSUE INJURY VOLUME ON CT BY THE LEVEL OF CD4 CELLS IN PATIENTS WITH CONFIRMED PULMONARY TUBERCULOSIS

Lung tissue injury volume according to chest CT	Level of CD4⁺ T-cells, cells/μL					
	< 50	50–99	100–199	200–349	350–499	> 500
Bilateral lesion (*n* = 84)	38 (45.2 %)	13 (15.5 %)	14 (16.7 %)	13 (15.5 %)	1 (1.2 %)	5 (5.9 %)
Destruction (*n* = 57)	23 (40.4 %)	9 (15.8 %)	8 (14.0 %)	8 (14.0 %)	1 (1.8 %)	8 (14.0 %)

TABLE 6

RESULTS OF DIAGNOSTIC METHODS OF MYCOBACTERIUM TUBERCULOSIS DETECTION

Methods (number of studies)	Number of positive results	
	abs. (*n* = 116)	%
Bacterioscopy of a smear with Ziehl – Neelsen staining (*n* = 396)	44	37.93
HAIN-GenoType MTBDRPlus (*n* = 396)	56	48.28
Bactec™ MGIT™ 960 (*n* = 396)	99	85.34
Inoculation of Löwenstein – Jensen dense medium (*n* = 396)	97	83.62

According to chest CT, bilateral lung lesion prevailed (45.2 %; *n* = 38) in patients with the level of CD4 cells < 50 cells/μL (Table 5). This group also had the largest number of cases of lung tissue destruction – 40.4 % (*n* = 23) – in patients who had not been examined for more than 3 years (16.2 %).

The diagnosis was considered verified in case of confirmation of MBT presence by various methods in 116 (66.6 %) patients (Table 6).

DISCUSSION

HIV-infected patients are a medical risk group for tuberculosis, with adolescents and adults required to undergo fluorography examination twice a year. In HIV-infected subjects, X-ray changes in the lungs with tuberculosis may be similar to other secondary and opportunistic diseases, making it difficult for radiologists to interpret the skiagram [3].

The manifestation and course of tuberculosis in patients with HIV infection differ significantly from its onset and course in subjects with a healthy immune system. As the number of $CD4^+$ lymphocytes decreases to a level of < 200 cells/μL, active fluorographic detection of respiratory tuberculosis is not justified; tuberculosis often has a generalized nature with synchronous lesions of several systems and organs. HIV-associated immunosuppression is characterized by a pleuropneumonia-like clinical picture, often with an acute onset, rapid and intensive disease development. The sensitivity of immunological tests also decreases in proportion to the degree of immunodeficiency.

When monitoring patients with HIV infection, difficulties in diagnosing tuberculosis often appear at stage 4. The level of immunosuppression is important in characterizing changes in the lungs. According to the majority of authors, when the level of CD4 lymphocytes is > 200 cells/μL, compared with the level of CD4 ≤ 200 cells/μL, the frequency of such radiological signs characteristic of the tuberculosis process during computed tomography of the chest organs as alveolar infiltration, destruction of lung tissue decreases, and the frequency of atypical manifestations increases (interstitial changes similar to "ground glass") [7]. According to the results of the study, all identified syndromes were assessed as characteristic of the tuberculosis process. In case of severe immunodeficiency, the volume of lung tissue injury increases in the form of a manifestation of bilateral dissemination and destruction, which indicates the process progression.

To detect pulmonary tuberculosis in adults, the most informative screening method is still plain lung radiography. Its insufficient information content in HIV-infected patients with severe immunosuppression can lead to the omission of pathology, especially with negative bacterioscopy data. Computed tomography as a more highly informative method allows to improve the quality, but given the high economic component for screening, it can be used differentially at the level of the AIDS center before referral to the TB dispensary, which can reduce the number of unjustified referrals and reduce the risks of infection of patients without tuberculosis and, conversely, accelerate the appointment of in-depth diagnostics in the specialized TB service. An integrated approach at the screening stages will prevent the development of progressive forms of tuberculosis in patients with HIV infection.

CONCLUSION

According to the Order of the Ministry of Health of the Russian Federation dated 21.03.2017 No. 124n "On approval of the procedure and timing of preventive medical examinations of citizens to detect tuberculosis", persons with HIV infection are classified to the group subject to preventive examination twice a year. To date, X-ray diagnostics in HIV-infected patients remains the key method of screening, forming a group for in-depth diagnosis of pulmonary tuberculosis. Computed tomography is mandatory if there is any doubt concerning the interpretation of changes on the plain lung radiography, especially in febrile HIV patients with severe immunosuppression in the absence of changes on the plain lung radiography of the chest organs and $CD4^+$ lymphocyte levels below 200 cells/μL.

Conflict of interest

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

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ASSESSMENT OF THE PSYCHO-EMOTIONAL STATE OF PATIENTS AFTER COVID-19-ASSOCIATED PNEUMONIA IN RELATIONSHIP WITH LABORATORY INDICATORS

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ABSTRACT

The aim. *To study peculiarities and association of psychological and laboratory indicators in patients with cardiovascular diseases (CVD) who underwent COVID-19 to clarify the factors affecting the possibility of developing delayed psychological and cardiovascular adverse events.*

Materials and methods. *The study enrolled 350 patients with COVID-19. Group 1 consisted of 92 patients without CVD, Group 2 – of 258 patients with CVD. Indicators of laboratory and psychological parameters were assessed according to the data of psychological questionnaire using GAD-7 (General Anxiety Disorder-7), PHQ-9 (Patient Health Questionnaire-9), PSS (Perceived Stress Scale) screening scales and SF-36. Parameters of complete blood count and biochemical blood tests were measured during hospitalization and three months after discharge from the monohospital.*

Results. *After three months, in the general group of patients, signs of anxiety and depression were detected in more than 30 % of the examined patients, signs of stress – in 10.4 %. In the group with CVD, psycho-emotional disorders were identified in 1/4 of the patients, and severe stress – in 8 % of those included in the study. In addition, it was registered that the indicators of erythrocyte sedimentation rate, fibrinogen, high-sensitivity C-reactive protein (CRP), homocysteine and IL-6 remained at a higher level in the second group. Correlation analysis showed that the psychological component of health is interconnected with the level of neutrophils ($p = 0.044$) and fibrinogen ($p = 0.050$); the physical component of health is correlated with the level of erythrocytes ($p = 0.030$), hemoglobin ($p = 0.015$), CRP ($p = 0.002$), creatine phosphokinase ($p = 0.036$) and glucose ($p = 0.017$). Regression analysis revealed that in patients with CVD three months after hospitalization, an increased glucose index contributes to deterioration, and increased hematocrit and mean hemoglobin concentration improve the quality of life of patients.*

Conclusion. *Laboratory markers that maintain the duration of a prolonged vascular reaction, violation of the rheological and metabolic properties of blood, determine the nature of the development of both psychological and cardiovascular complications.*

Key words: *COVID-19, biomarkers, psychological status, cardiovascular disease*

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ОЦЕНКА ПСИХОЭМОЦИОНАЛЬНОГО СОСТОЯНИЯ ПАЦИЕНТОВ, ПЕРЕНЁСШИХ COVID-19-АССОЦИИРОВАННУЮ ПНЕВМОНИЮ, ВО ВЗАИМОСВЯЗИ С ЛАБОРАТОРНЫМИ ПОКАЗАТЕЛЯМИ

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РЕЗЮМЕ

Цель. *Исследовать особенности и ассоциацию психологических и лабораторных показателей у пациентов с сердечно-сосудистыми заболеваниями (ССЗ), перенёсших COVID-19-ассоциированную пневмонию, с целью уточнения факторов, влияющих на возможность развития отсроченных психологических и сердечно-сосудистых нежелательных явлений.*

Материалы и методы. *В исследование включены 350 пациентов, сформировано две группы исследования. Первая группа – 92 пациента без ССЗ, вторая – 258 пациентов с ССЗ. Оценивали параметры общего и биохимического анализов крови, психологические показатели по данным анкетирования с использованием скрининговых шкал GAD-7 (General Anxiety Disorder-7 – признаки тревоги), PHQ-9 (Patient Health Questionnaire-9 – признаки депрессии) и ШВС (шкала воспринимаемого стресса – признаки стресса) и опросника качества жизни (SF-36, Short Form 36) во время госпитализации и через 3 месяца после выписки из моногоспиталя.*

Результаты. *Через 3 месяца в общей группе пациентов признаки тревоги и депрессии выявлены более чем у 30 % обследованных пациентов, признаки стресса – у 10,4 %. В группе с наличием ССЗ нарушения психоэмоциональной сферы определены у 1/4 больных, а выраженный стресс – у 8 % включённых в исследование. Кроме этого, зарегистрировано, что показатели скорости оседания эритроцитов, фибриногена, высокочувствительного С-реактивного белка (СРБ), гомоцистеина и ИЛ-6 сохранились на более высоком уровне во второй группе. Корреляционный анализ показал, что психологический компонент здоровья взаимосвязан с уровнем нейтрофилов ($p = 0,044$) и фибриногена ($p = 0,050$); физический компонент здоровья взаимосвязан с уровнем эритроцитов ($p = 0,030$), гемоглобина ($p = 0,015$), СРБ ($p = 0,002$), креатинфосфокиназы ($p = 0,036$) и глюкозы ($p = 0,017$). Регрессионный анализ выявил, что у пациентов с ССЗ через 3 месяца после госпитализации повышенный показатель глюкозы способствует ухудшению, а повышенные показатели гематокрита и средней концентрации гемоглобина – улучшению качества жизни пациентов.*

Заключение. *Лабораторные маркеры, поддерживающие длительность пролонгированной сосудистой реакции, нарушение реологических и метаболических свойств крови, определяют характер развития как психологических, так и сердечно-сосудистых осложнений.*

Ключевые слова: *COVID-19, психологический статус, биомаркеры, сердечно-сосудистые заболевания*

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INTRODUCTION

The pandemic of coronavirus infection COVID-19 (COronaVIrus Disease-2019), which is caused by a new strain of coronavirus – SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2), has caused a rapid increase in the number of cases and high mortality worldwide [1]. Despite the tropism of SARS-CoV-2 to the lungs, COVID-19 has a high risk of developing multiple organ failure (MOF), including due to cardiovascular system (CVS) diseases [1, 2]. The expression of receptors in the vascular wall, cardiomyocytes makes it possible to partially explain the pathogenetic basis of the occurrence and features of the course of cardiovascular diseases, and the effect of the virus on the central nervous system determines the development of neurological and psychological disorders [3, 4].

At the beginning of the pandemic, there was no information about the long-term consequences of this infectious disease, but as the course of the process was studied, more and more data appeared in the literature regarding an expanded spectrum of systemic, cardiovascular, neurological and psychosocial symptoms. The authors described symptoms of varying duration from 1.5 months to 1 year from the onset of the acute stage of the disease, and there was not always a correlation between the severity of COVID-19, the number and severity of delayed manifestations [5]. It is assumed that the mechanisms underlying the post-COVID syndrome include changes in the immune response and damage to the vascular bed with the development of hypercoagulable thrombotic complications [6]. The entry point into the central nervous system for the virus can be either indirect – through the bloodstream, or direct – through the lattice plate. Moreover, it is assumed that the infection can cause a general depression of the hypothalamus-pituitary axis, associated with the systemic effect of inflammation and hypoxia [7]. Post-treatment follow-up is necessary not only to understand the relationship between the manifestations of the post-COVID syndrome and the course of the disease caused by the SARS-CoV-2 virus, but also to justify the need to develop algorithms for effective prevention of post-covid complications, including programs for restoring psychological health.

THE AIM OF THE STUDY

To study peculiarities and association of laboratory and psychological indicators in patients with cardiovascular diseases who have underwent COVID-19 to optimize preventive measures against the development of delayed cardiovascular and psychological adverse events.

MATERIALS AND METHODS

Prospective study complying with the standards of Good Clinical Practice and the provisions of the Helsinki Declaration of the World Medical Association. Research protocol was approved by the Committee on Biomedical Ethics of the Tyumen Cardiology Research Center – Branch of the Tomsk National Research Medical Center of the Russian Academy of Sciences (Protocol No. 159 dated July 23, 2020). Prior to enrollment in the study, each of the study participants received written informed consent to use the survey results for scientific purposes. The study is registered in the clinical research database ClinicalTrials.gov (identifier: NCT04501822).

Patients were identified according to the data of the 1C medical information system of the monoinfective hospital on the basis of the Tyumen Regional Clinical Hospital in the period from April 10, 2020 till July 11, 2021. Data on diagnoses and examination results during the hospitalization of patients in the monohospital are taken from the extracts provided by patients from medical records and 1C system data. The inclusion criteria were: a documented diagnosis of COVID-19 associated pneumonia and the patient's desire to participate in the follow-up. Criteria for non-inclusion: chronic and systemic diseases in the acute stage; diseases accompanied by pneumofibrosis; oncological diseases detected less than 5 years ago. Exclusion criteria: pregnancy, refusal to participate in the study.

The study included 350 patients who agreed to follow-up. The patients were divided into two groups. The first group included 92 patients without cardiovascular diseases (CVD), the average age was 42.16 ± 11.18 years. The second group included 258 patients with CVD (arterial hypertension (AH) and coronary heart disease (CHD)), the average age was 56.30 ± 8.44 years. There were statistically significant differences in age, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), body mass index (BMI) with higher indicators in the second group ($p < 0.001$). The differences of the listed parameters between the groups did not affect the study of the parameters dynamics within the groups. There were no statistically significant differences by gender in the study groups ($p < 0.312$), women accounted for 56.5 % (52 patients) in the first group and 50.4 % (130 patients) in the second. According to the chest computed tomography data, initially in the first group the volume of lung lesion of more than 50 % was registered in 30.7 % of cases, in the second group – in 57.8 % of cases.

With respect to CVDs in the second group, 98 % of patients had hypertension, 20.5 % had coronary artery disease, 32.6 % had cardiac arrhythmias, etc. By the time of the control visit after 3 months, patients in the second group (with CVDs) in 22.4 % of cases were taking drugs of angiotensin-converting enzyme inhibitors (ACE inhibitors), in 48.2 % of cases – angiotensin receptor blockers (ARBs), in 46.6 % of cases – beta blockers, in 61.7 % of cases – statins, in 19.8 % – acetylsalicylic acid preparations, in 7.51 % – oral anticoagulants.

The study evaluated the data of laboratory tests of blood samples taken during hospitalization and at the point 3 months after discharge from the monohospital. The parameters of the Complete Blood Count (CBC) were determined by the impedance method with flow cytome-

try technologies on 5Diff analyzer "Mindrey BC 5800" (China); creatinine, liver enzymes, total cholesterol, fasting glucose, concentration of C-reactive protein (CRP) – "Cobas integra plus 400" (Italy), reagents of Mindrty were studied from biochemical parameters; highly sensitive CRP (hs-CRP) – solid-phase enzyme immunoassay, Vector Best reagents (Russia); interleukin-6 (IL-6) – solid-phase, chemiluminescent enzyme immunoassay, IMMULITE 1000 Systems SIEMENS Healthcare Diagnostics (Germany); and homocysteine – on the analyzer "IMMULITE 2000" (Siemens Diagnostics, USA), solid-phase, competitive, chemiluminescent enzyme immunoassay, reagents from SIEMENS Healthcare Diagnostics (Germany); coagulogram parameters – on Destiny Plus analyzer (Ireland), reagents from NPO Renam (Russia). The psycho-emotional sphere of patients was studied using the screening scales GAD-7 (General Anxiety Disorder-7 – signs of anxiety), PHQ-9 (Patient Health Questionnaire-9 – signs of depression) and SPS (scale of perceived stress – signs of stress). For anxiety and depression scales, the threshold value for the presence of signs of disorders was considered to be 5 scores, for signs of severe stress – 30 scores. The quality of life (QOL) was assessed using SF-36 questionnaire, which allows us to characterize this concept quantitatively according to 8 specific and 2 generalized scales showing psychological and physical health. The physical health includes Physical functioning, Role-based physical functioning, Pain intensity and overall health status scales. The psychological health consists of Mental health, Role-based emotional functioning and Vitality scales. In this paper, we relied on the indicators of generalized scales [8–10].

Statistical analysis was carried out using IBM SPSS Statistics 21 application software package (IBM Corp., USA). Depending on the distribution, when comparing the indicators in two independent groups, the Student's t-test or Mann – Whitney U test were used. With a normal distribution, the data were presented as mean (M) and standard deviation (SD), with a distribution other than normal, the data were presented as median (Me) and interquartile range [25 %; 75 %]. The dynamics between related groups was calculated using the paired Student t-test or the Wilcoxon test. Spearman and Pearson correlation analysis (depending on the type of data distribution) and linear regression with step-by-step inclusion of predictors in the model were used. The purpose of the regression analysis was to identify the predictors that have the greatest impact on the psycho-emotional state of patients with CVDs who have suffered COVID-19 pneumonia. The results were evaluated as statistically significant at the two-sided $p < 0.05$ level.

RESULTS

The analysis of the parameters of the complete blood count in the study groups of patients is given in Table 1.

Analysis of the table data showed that individual erythrocyte parameters, such as RDW-SD, RDW-CV and ESR, in the second group of patients exceeded the ones in the first group ($p < 0.001$, $p < 0.023$ and $p < 0.001$, respectively).

The remaining parameters varied in different directions in both groups, remaining within the reference values to the observation point "3 months after discharge from the hospital". The level of ESR decreased statistically significantly in the second group of patients ($p < 0.001$).

The characteristics of leukocyte parameters in the patients included in the study are given in Table 2.

The data show that at baseline there was a statistically significant increase in WBS, NLR and a decrease in the LYM/CRP ratio in patients of the second group ($p < 0.001$).

Three months after admission, there were statistically significant decreases in WBC, NEU, NLR ratio and increases in EOS, LYM/CRP in both groups and LYM in the second group of patients. However, the parameters of WBC, LYM, NEU, while remaining statistically significantly higher than in the first group, indirectly confirm the presence of a persistent prolonged inflammatory reaction in patients with CVDs caused by the coronavirus infection.

According to platelet counts, no statistically significant difference was found at the baseline of the study. Three months after discharge, a statistically significant decrease in the mean platelet volume (MPV) was registered: baseline in the first group – 11.20 [10.70; 11.9] fL, in dynamics – 8.40 [7.90; 8.90] fL, in the second group – 11.20 [10.50; 11.70] and 8.30 [7.70; 11.70] fL, respectively ($p < 0.001$ for both groups); platelet large cell count (PLCC): baseline in the first group – 35.00 [30.20; 41.60] %, in dynamics – 23.80 [19.30; 29.70] %, in the second group – 34.30 [28.80; 37.70] and 23.80 [19.30; 28.80] %, respectively ($p < 0.001$ for both groups); statistically significant increase in platelet count (PLT): baseline in the first group – 206.00 [152.00; 258.00], in dynamics – 226,00 [190,00; 261,00] $\times 10^9$/L, in the second group – 203.50 [156.50; 250.50] and 230,00 [200,00; 272,00] $\times 10^9$/L, respectively ($p < 0.001$).

Of the hemostasis parameters, patients in the second group had a statistically significant fibrinogen level increase at baseline compared to the first group (4.60 [3.60; 5.80] and 3.90 [3.60; 5.80] g/L, respectively; $p < 0.001$) with achievement of reference values in both patient groups in 3 months. In addition, increased fluctuations of these parameters of APTT and PTI were recorded in the second patient group.

At baseline, characterization of biochemical parameters in the groups showed an excess of fasting glucose in both groups (6.70 [6.22; 7.39] and 7.52 [6.79; 9.40] mmol/L, respectively) with a statistically significant decrease in the parameters after 3 months ($p < 0.001$, respectively, for the groups) and a statistically significant excess of HbA1c (6.70 [5.90; 8.00] in the second group); excess values of aspartate aminotransferase (AST) (32.80 [23.45; 50.80] U/L), alanine aminotransferase (ALT) (34.20 [22.90; 53.20] U/L) and total cholesterol (TCL) (4.11 [3.32; 4.84] mmol/L) in the second patient group. In addition, there was a statistically significant excess of baseline lactate dehydrogenase (LDH) values (361.14 ± 135.98 and 437.50 ± 212.85 U/L) and max CRP (29.8 [3.80; 24.60]

TABLE 1

COMPARATIVE CHARACTERISTICS OF ERYTHROCYTE PARAMETERS IN PATIENTS WITH THE ABSENCE AND PRESENCE OF CARDIOVASCULAR DISEASES WHO SUFFERED COVID-19 PNEUMONIA, AT BASELINE AND THREE MONTHS AFTER HOSPITAL ADMISSION

Parameters	Examination period	Without CVDs (*n* = 92)	With CVDs (*n* = 258)	*p*
RBC, 10^{12}/L (norm: male – 4.0–5.2; female – 3.9–4.7)	At baseline	4.79 ± 0.49	4.71 ± 0.54	0.200
	In 3 months	4.76 ± 0.43	4,90 ± 0,44	**0.003**
	p	0.151	**< 0.001**	
HGB, g/L (norm: male – 130–166; female – 117–140)	At baseline	137.50 [125.50; 146.50]	137.00 [127.00; 146.00]	0.770
	In 3 months	136.00 [127.00; 146.00]	142.00 [132.00; 149.00]	**0.008**
	p	0.852	**< 0.001**	
HCT, % (norm: male – 39–49; female – 35–43)	At baseline	40.20 [37.80; 43.10]	40.50 [37.50; 43.00]	0.968
	In 3 months	43.70 [40.80; 46.00]	45.40 [42.60; 48.10]	**< 0.001**
	p	**< 0.001**	**< 0.001**	
MCV, fL (norm: 80–95)	At baseline	85.10 [80.40; 87.20]	85.00 [82.20; 88.50]	0.095
	In 3 months	92.00 [88.00; 94.00]	92.00 [90.00; 95.00]	**0.039**
	p	**< 0.001**	**< 0.001**	
MCH, pg (norm: 27–31)	At baseline	28.90 [27.70; 29.70]	29.00 [27.90; 30.00]	0.272
	In 3 months	29.00 [28.00; 30.00]	29.00 [28.00; 30.00]	0.903
	p	**0.055**	0.876	
MCHC, g/dL (norm: 31–37)	At baseline	33.60 [33.10; 34.30]	33.80 [33.20; 34.50]	0.360
	In 3 months	32.00 [31.00; 32.00]	31.00 [31.00; 32.00]	**0.001**
	p	–	–	
RDW–SD, fL (norm: 35–56)	At baseline	39.50 [37.50; 40.90]	40.50 [38.80; 43.00]	**0.001**
	In 3 months	46.40 [44.30; 48.00]	48.50 [46.30; 51.30]	**< 0.001**
	p	**< 0.001**	**< 0.001**	
RDW–CV, % (norm: 11.5–14.5)	At baseline	13.00 [12.40; 13.60]	13.30 [12.80; 13.90]	**0.023**
	In 3 months	12.40 [12.00; 12.80]	12.90 [12.30; 13.60]	**< 0.001**
	p	**< 0.001**	**< 0.001**	
ESR, mm/h (norm: 0–15)	At baseline	12.50 [8.00; 16.00]	39.00 [24.00; 53.00]	**< 0.001**
	In 3 months	11.00 [7.00; 17.00]	12.00 [8.00; 19.00]	0.327
	p	0.600	**< 0.001**	

Note. RBC – red blood cells; HGB – hemoglobin (concentration); HCT – hematocrit; MCV – mean corpuscular/cell volume; MCH – mean corpuscular haemoglobin; MCHC – mean cell haemoglobin concentration; RDW-SD – red cell distribution width, standard deviation); RDW-CV – red cell distribution width, coefficient of variation/variation coefficient; ESR – erythrocyte sedimentation rate. *p* is the statistical significance of the differences in parameters: horizontally – between the first and second groups; vertically – within the group in dynamics – at baseline and 3 months after discharge from the hospital.

TABLE 2

COMPARATIVE CHARACTERISTICS OF LEUKOCYTE PARAMETERS IN PATIENTS WITH THE ABSENCE AND PRESENCE OF CARDIOVASCULAR DISEASES WHO SUFFERED FROM COVID-19 PNEUMONIA, AT BASELINE AND 3 MONTHS AFTER HOSPITAL ADMISSION

Parameters	Examination period	Without CVDs (*n* = 92)	With CVDs (*n* = 258)	*p*
WBS, 10^9/L (norm: 4.0–8.8)	At baseline	4.98 [3.88; 6.70]	6.53 [4.92; 8.13]	**< 0.001**
	In 3 months	4.56 [4.04; 5.72]	5.65 [4.81; 6.78]	**< 0.001**
	p	**0.050**	**< 0.001**	
LYM, 10^9/L (norm: 1,4–8)	At baseline	1.43 [1.01; 1.95]	1.23 [0.88; 1.67]	**0.035**
	In 3 months	1.58 [1.41; 1.90]	1.92 [1.61; 2.32]	**< 0.001**
	p	**0.011**	**< 0.001**	
NEU, 10^9/L (norm: 1.8–7.7)	At baseline	2.94 [2.02; 4.43]	4.37 [2.98; 6.29]	**< 0.001**
	In 3 months	2.52 [2.04; 3.16]	3.05 [2.38; 3.81]	**< 0.001**
	p	**0.010**	**< 0.001**	
EOS, 10^9/L (norm: 0–5)	At baseline	0.03 [0.01; 0.10]	0.02 [0.01; 0.06]	**0.025**
	In 3 months	0.10 [0.06; 0.16]	0.12 [0.08; 0.18]	**0.048**
	p	**< 0.001**	**< 0.001**	
NLR (norm: 1.6–1.8)	At baseline	1.96 [1.30; 3.09]	3.47 [2.03; 6.18]	**< 0.001**
	In 3 months	1.57 [1.17; 2.06]	1.57 [1.17; 2.00]	0.659
	p	**0.004**	**< 0.001**	
LYM/CRP	At baseline	0.11 [0.02; 0.38]	0.02 [0.01; 0.04]	**< 0.001**
	In 3 months	0.51 [0.32; 1.63]	0.39 [0.26; 0.74]	**0.005**
	p	**< 0.001**	**< 0.001**	

Note. WBS – white blood cells; LYM – the number of lymphocytes; NEU – the number of neutrophils; EOS – the number of eosinophils; NLR – the ratio of neutrophils to lymphocytes; LYM/CRP – the ratio of leukocytes to C-reactive protein. *p* is the statistical significance of the differences in parameters: horizontally – between the first and second groups; vertically – within the group in dynamics – at baseline and 3 months after discharge from the hospital.

and 65.10 [32.00; 128.00 mg/L]) in both groups with a decrease in the parameters after 3 months with a continued value excess in the second group ($p < 0.001$, respectively).

It is important to note that during the planned complex drug therapy performed 3 months after discharge, the second group showed increased levels of homocysteine, IL-6, and hs-CRP (14.36 ± 6.08 and 14.67 ± 6.69 mmol/L), (1.75 [1.30; 2.45] and 2.26 [1.60; 3.90] μg/ml), (2.13 ± 2.26 and 4.13 ± 3.92 mg/L), exceeding both the reference values and the indicators in the first group. The recorded parameters related to the planned complex drug therapy indicate the presence of increased vascular inflammatory potential for the development of possible adverse events in patients during the post-COVID period.

Simultaneously with blood sampling at the point "3 months after hospital discharge", a medical psychologist collected questionnaire details from all patients reflecting the psycho-emotional state of patients who had un-

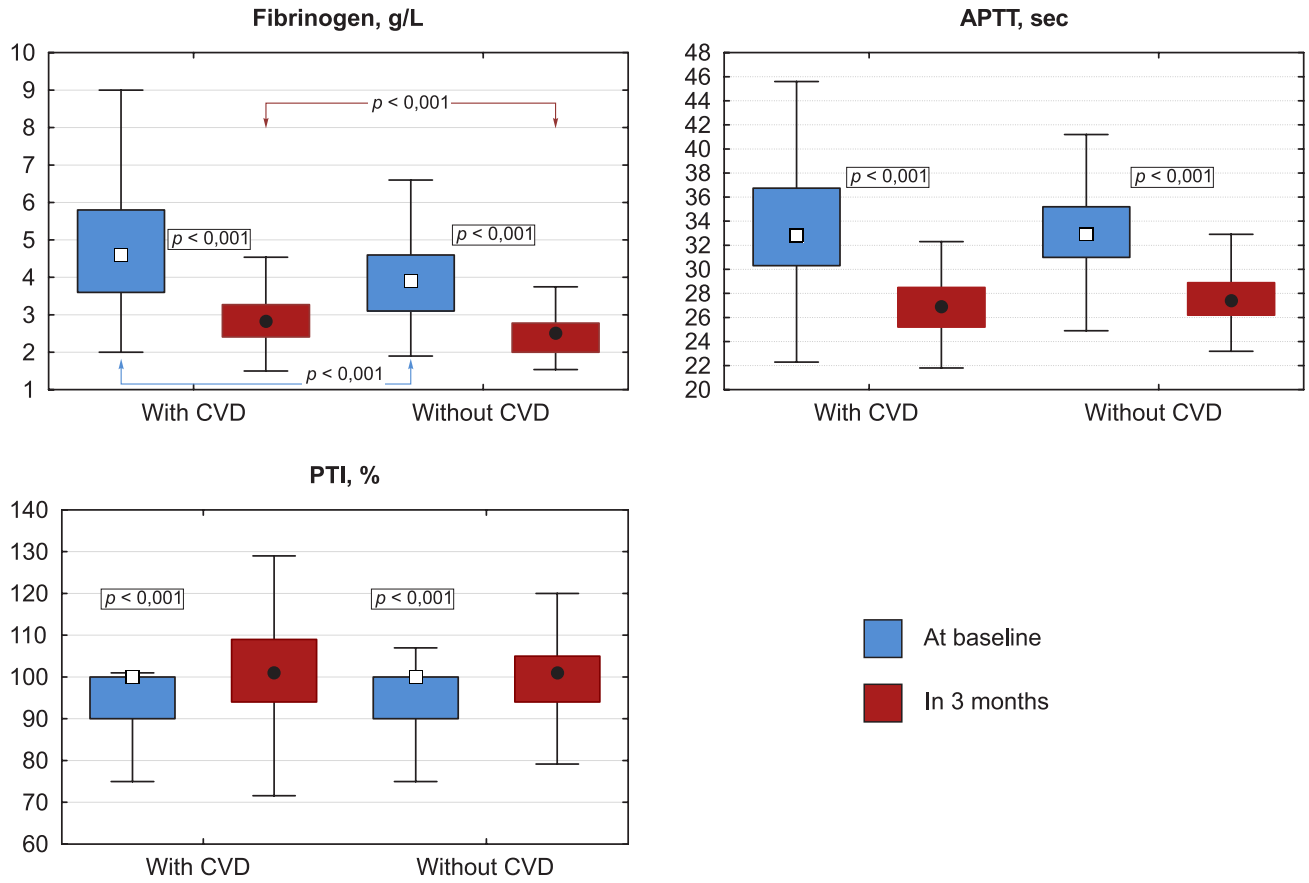

FIG. 1.

Hemostasis parameters in patients with and without cardiovascular disease who had COVID-19 pneumonia at baseline and three months after hospital admission

TABLE 3

COMPARATIVE CHARACTERISTICS OF THE SEVERITY OF DISORDERS OF THE PSYCHO-EMOTIONAL SPHERE AND QUALITY OF LIFE IN PATIENTS WITH THE ABSENCE AND PRESENCE OF CARDIOVASCULAR DISEASES WHO SUFFERED FROM COVID-19 PNEUMONIA, THREE MONTHS AFTER HOSPITAL ADMISSION

Parameters	Without CVD, Me [25%; 75%]	With CVD, Me [25%; 75%]	*p*
Signs of anxiety	3.00 [1.00; 6.00]	2.00 [1.00; 6.00]	0.770
Signs of depression	3.00 [1.00; 5.00]	3.00 [1.00; 6.00]	0.507
Signs of stress	20.00 [15.00; 25.00]	21.00 [15.00; 26.00]	0.300
The physical health	50.27 [47.31; 52.72]	46.88 [41.33; 50.88]	**0.000**
Psychological health	67.08 [57.17; 71.18]	65.55 [57.66; 72.01]	0.748

dergone COVID-19. Screening scales GAD-7 (signs of anxiety), PHQ-9 (signs of depression), SHVS (signs of stress) and SF-36 questionnaire were used to assess patients' quality of life. The data of general characterization of the psychological sphere of patients showed that in the general group of patients, signs of anxiety and depression were detected 3 months after hospital discharge in more than 30 % of the examined patients, signs of stress – in 10.4 %. In the group of patients with CVDs, disorders of the psycho-emotional sphere were found in 1/4 of the patients en-

TABLE 4

RESULTS OF REGRESSION ANALYSIS OF THE EFFECT OF BLOOD PARAMETERS IN PATIENTS WITH CARDIOVASCULAR DISEASES ON THE PSYCHO-EMOTIONAL STATE AND QUALITY OF LIFE THREE MONTHS AFTER HOSPITAL DISCHARGE

Parameters	Predictors	Non-standardized coefficient b	Standardized coefficient β	*p*
Severity of anxiety	Thrombocrit	17.855	0.226	0.014
	Fibrinogen	1.700	0.256	0.007
Severity of depression	LDH	0.030	0.226	0.015
	Ferritin	–0.008	–0.203	0.031
Severity of stress	Fibrinogen	3.002	0.265	0.003
	Glucose (acute period)	–0.727	–0.356	0.003
The physical health	Hematocrit	0.326	0.188	0.002
	Average hemoglobin concentration	1.068	0.148	0.017

rolled in the study. Severe stress is typical for 8 % of those included in the study.

When comparing the severity of disorders of the psycho-emotional sphere and generalized indicators of QOL (SF-36), the results of only the physical health differ statistically significantly between groups of patients with the absence and presence of CVD. The data is shown in Table 3.

The physical health was statistically significantly reduced in the group of CVD patients, which is consistent with the clinical data on the presence of an increase in the frequency of crisis states of hypertension, newly detected cases of coronary heart disease (6.1 %), chronic heart failure (8.7 %) and 1 case of diabetes mellitus in this group. There were no differences between the groups by categories of psycho-emotional state.

The relationship of the psychological state of CVD patients and hematological and biochemical blood parameters is shown by the results of correlation analysis. During admission, the psychological health is inversely correlated with the level of neutrophils ($r = –0.137$; $p = 0.044$) and directly correlates with the level of fibrinogen ($r = 1.135$; $p = 0.050$); the physical health is interrelated with the level of erythrocytes ($r = 0.140$; $p < 0.030$), hemoglobin ($r = 0.158$; $p = 0.015$), CRP ($r = –0,200$; $p = 0.002$), CPK ($r = 0.175$; $p = 0.036$) and glucose ($r = –0,182$; $p = 0.017$). In the post-COVID period (3 months after discharge), the psychological health was associated with the level of transforming growth beta factor ($r = 0.404$; $p = 0.030$); the physical health was associated with the level of erythrocytes ($r = 0.143$; $p = 0.023$), hemoglobin ($r = 1.222$; $p = 0.001$), hematocrit ($r = 0.187$; $p = 0.003$). During general data processing, it was recorded that the level of anxiety and depression was more related to hematological indicators, while the level of stress was more related to the level of inflammatory parameters. The results of the regression analysis aimed at determining blood biomarkers that affect the psycho-emotional state and CVD patients's quality of life are given in Table 4.

The results of the regression analysis indicate that an increase in the level of anxiety in patients with cardiovascular diseases is associated with an increase in the blood thrombocrit level. Depression scores increase statistically significantly with an increase in fibrinogen and LDH levels and a decrease in ferritin levels. The severity of stress is affected by changes in the level of fibrinogen.

In addition, the analysis showed that in patients with cardiovascular diseases, 3 months after hospital admission, the physical health has an inverse relationship with glucose levels in the acute period of the disease. Increased glucose levels contribute to a prolonged deterioration of the physical component of QOL, especially in patients with baseline elevated glucose levels. Increased hematocrit and average hemoglobin concentration after discharge add scores to the physical health component, which indicates an improvement in patients' quality of life.

DISCUSSION

Based on a number of studies, patients who have undergone COVID-19 often show altered parameters of lab-

oratory findings in the post-COVID period, the relationship between which and newly emerged clinical manifestations may indicate the presence of a latent prognostic potential in the development of delayed cardiovascular events [11–18].

Most of the published results have been confirmed in our works and our own data have been obtained, in particular, on the association of the volume of lung tissue lesion with the parameters of erythrocyte and leukocyte formulas, coagulation profile, liver enzymes at the initial stage of the disease, on the influence of inflammatory markers and hyperglycemia on the risk of adverse vascular complications of the disease [20]. Thus, lymphopenia and eosinopenia have prognostic significance in the detection of a severe course of the disease and hospital mortality [13, 14]. Registered microangiopathies and microthrombosis in hepatic sinusoids are associated with impaired hepatic enzyme levels, with elevated bilirubin levels and hyperglycemia associated with the persistent inflammatory process in hepatocytes and cholangiocytes [15–20].

The problem of psychological consequences of COVID-19 is the object of attention. Many authors have noted a significant degree of heterogeneity in terms of populations, sampling methods, and scales in such studies, which complicates data interpretation [21, 22]. Dozens of meta-analyses are devoted to physical and mental health. For example, in Europe, more than 3 million people participated in 692 primary studies where it was shown that the prevalence of psychological problems was closely related to the patients themselves, their environment and social support. The findings clearly indicate that social isolation is associated with a number of adverse consequences for physical and mental health [23–25].

The research results published by Chinese experts showed that 53.8 % of respondents who underwent COVID-19 assessed the registered individual psychological manifestations as protective factors. Anxiety is designed to perform an adaptive function in stressful circumstances, which in a pandemic are represented by social aspects of isolation and distancing, the use of antiseptics, as well as concern about health, future and material well-being. Functional level anxiety motivates a person to look for ways to solve problems, change habitual behavior, which allows patients to strengthen their faith in the competence of physicians and reports on successful recovery, having a mobilizing effect on a person. However, the presence of severe anxiety negatively affects cognitive functioning and coping behavior aimed at overcoming problematic situations [21].

The level of psychological stress is more often associated with a deterioration in social functioning: a decrease or lack of support, the detection of COVID-19 in relatives or the loss of ones due to infection, as well as the presence of a non-transmissible disease that significantly worsens somatic health. Overstress is often combined with symptoms of anxiety or depression, which seriously impairs a person's adaptive abilities.

According to the results of research by Russian scientists, severe deterioration in both physical and emotional health has been recorded in the post-reproductive-stage women after suffering a moderately severe form of COVID-19. The results suggest a potential link between COVID-19 and future risk of cognitive decline, persistent deterioration of health and quality of life [23].

Depressive states are often a response to changes in social status, marital status, and material well-being. After COVID-19, factors contributing to persistent mood declines include persistent physiological manifestations of the disease (prolonged loss of taste and smell, sleep disorders, increased heartbeat and fatigue), peculiarities of disease perception and behavior (frequent news viewing and isolation after hospital discharge) [22].

It is known that patients with cardiovascular diseases are characterized by high comorbidity with disorders of the anxiety and depressive spectrum, which not only worsens the severity of the course and prognosis of the disease, but also contributes to a decrease in the effectiveness of the therapy received by the patient. In this regard, it is recommended to influence psychosocial factors with the use of pharmacological therapy and psychological correction in this category of patients [26].

During the study, the data on the study of neuroimmunological processes and their association with proinflammatory cytokines in the pathogenesis of anxiety-depressive disorders were of particular interest to us [21, 27].

Intensely perceived stress leads to activation of the autonomic nervous system and hypothalamic-pituitary-adrenal axis, resulting in increased levels of cortisol, adrenaline, norepinephrine. This in turn enhances the inflammatory response by inhibiting humoral and cellular immunity, and changes the balance of pro-inflammatory cytokines. At the same time, SARS-CoV-2 infection leads to the production of IL-Ib and IL-6 [27]. The works of other authors confirm the relationship between fibrinogen and stress, which leads to the formation of endothelial dysfunction and deterioration of cardiovascular health [28]. It is also known that the presence of anxiety states is associated with increased levels of platelet counts [29]. The relationship between fibrinogen, depression and CVD has also been described, where researchers consider fibrinogen as the key factor in the formation of cardiovascular disorders, since this marker contributes to increased platelet aggregation and blood clotting, which increases plasma viscosity and thrombosis [30]. In addition, the literature describes the relationship of depressive states with ferritin, which is a biomarker of the iron amount in the blood. H.S. Lee et al. found a relationship between ferritin, depression, and overweight, which aggravates the course of CVD [31]. The effect of glucose and the consequences of the formation of excessive amounts of metabolically active free oxygen radicals in the acute period of COVID-19 on the deterioration of the physical aspect of QOL is of interest [32]. In addition, we should not forget about the possible mechanisms of drug-induced lesion processes of the target organs and systems, modifying both psychological and physical components of health [21, 25].

The decrease in the level of proinflammatory cytokines IL-1ß, tumor necrosis factor α, responsible for depressive-

like behavior recorded in animal models by antidepressants broadens the research horizons of possible prevention and complex therapy of patients with viral infections in the future [21].

Study limitations

In this paper, only generalized data on the psychological and physical components of health were studied. In the future, detailed characteristics of the studied data will be described, taking into account age, gender, and social characteristics.

CONCLUSION

Analysis of the literature data and our own research results suggest that the detected changes in biomarkers and the presence of their relationship with psychological manifestations allow us to consider the expectation of an increase in anxiety-depressive disorders in COVID-19 survivors justified, determining the relevance of the medical problem under study. The lack of data in terms of the common links in the pathogenesis of the investigated combined states allows, through the study of laboratory markers, to identify a range of parameters that together can both initiate and maintain the duration of a prolonged vascular reaction that determines the risk of developing both cardiovascular and neuropsychiatric complications.

Our findings indicate the need for dynamic follow-up of patients and the feasibility of developing preventive measures optimized by specialized specialists, both for the post-COVID period and for future waves of the pandemic and subsequent daily life.

Conflict of interest

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

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THE ROLE OF TOLL-LIKE RECEPTOR 4 GENE POLYMORPHISM IN THE DEVELOPMENT OF ORGAN DYSFUNCTION IN PATIENTS WITH SEVERE PNEUMONIA ASSOCIATED WITH A/H1N1 INFLUENZA

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ABSTRACT

The aim. *To identify the frequency of occurrence of TLR4 Asp299Gly (rs4986790) gene polymorphism and to establish its contribution to the development of organ dysfunction in patients with severe pneumonia associated with A/H1N1 influenza.*
Materials and methods. *The study included 55 patients with severe pneumonia associated with A/H1N1 influenza. Inclusion criteria: severe pneumonia; consolidation/ground-glass syndrome according to chest X-ray/CT. Exclusion criteria: unstable hemodynamics; body mass index > 30; diabetes mellitus; HIV; tuberculosis, oncopathology. Verification of the pathogen in the respiratory swab was carried out using PCR method: A/H1N1 influenza virus RNA was identified. The age of the patients was 47 [38; 62] years. Among all the patients the proportion of men was 47.8 %, of women – 52.2 %. Patients were divided into 2 groups: group 1 included patients with SOFA scale (Sequential Organ Failure Assessment) score ≥ 2 points; group 2 – patients with SOFA scale score < 2 points. Gene SNPs were determined by PCR method using standard kits developed by Research and Production Company "Litekh" (Moscow). Amplification of the TLR4 gene fragments was carried out in a thermocycler Bis-M111 (Bis-N LLC, Novosibirsk). Genomic DNA isolated from whole blood leukocytes using the "DNA Express Blood" reagent was analyzed followed by an amplification reaction. The amplification product was detected in a 3% agarose gel.*
Results. *Multiple organ dysfunction (SOFA scale score ≥ 2 points) in patients with severe pneumonia associated with A/H1N1 influenza was registered in 24 (43.6 %) cases. When analyzing the frequency of occurrence of the minor Gly allele, according to genetic models, the differences were established between patients of the groups 1 and 2 in codominant (p = 0.023; odds ratio (OR) – 8.82 (0.95–81.89)) and dominant (p = 0.005; OR = 12.35 (1.40–109.07)) models.*
Conclusion. *Severe pneumonia associated with A/H1N1 influenza is accompanied by a high incidence of organ dysfunction. The risk of organ failure development is 2.1 times increased in patients with severe pneumonia with identified TLR4 Asp299Gly gene polymorphism, which probably requires further study.*

Key words: *TLR4, polymorphism, A/H1N1 influenza, pneumonia, organ dysfunction*

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РОЛЬ ПОЛИМОРФИЗМА ГЕНА TOLL-ПОДОБНОГО РЕЦЕПТОРА 4 В РАЗВИТИИ ОРГАНОЙ ДИСФУНКЦИИ У БОЛЬНЫХ ТЯЖЁЛОЙ ПНЕВМОНИЕЙ ПРИ ГРИППЕ A/H1N1

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РЕЗЮМЕ

Цель исследования. *Выявить частоту встречаемости полиморфизма гена TLR4 Asp299Gly (rs4986790) и установить его вклад в развитие органной дисфункции у больных тяжёлой пневмонией на фоне гриппа A/H1N1.*

Материалы и методы. *В исследование включено 55 больных тяжёлой пневмонией на фоне гриппа A/H1N1. Критерии включения: пневмония тяжёлого течения; наличие консолидации/синдрома «матового стекла» по данным рентгенографии/компьютерной томографии органов грудной клетки. Критерии исключения: нестабильная гемодинамика; индекс массы тела > 30; сахарный диабет; ВИЧ; туберкулёз; онкопатология. Верификация возбудителя в респираторном мазке выполнялась при помощи метода полимеразной цепной реакции (ПЦР): идентифицирована РНК вируса гриппа A/H1N1. Возраст пациентов составил 47 [38; 62] лет. Мужчин было 47,8 %, женщин – 52,2 %. Пациенты были поделены на две группы: 1-я группа – с оценкой по шкале SOFA (Sequential Organ Failure Assessment) ≥ 2 баллов; 2-я группа – с оценкой по шкале SOFA < 2 баллов. Определение однонуклеотидных полиморфизмов (SNP, single nucleotide polymorphism) генов осуществлялось методом ПЦР с использованием стандартных наборов НПФ «Литех» (Москва). Амплификацию фрагментов гена TLR4 проводили в термоциклере «Бис-М111» (ООО «Бис-Н», Новосибирск). Анализу подвергалась геномная ДНК, выделенная из лейкоцитов цельной крови с помощью реагента «ДНК экспресс-кровь», затем проводилась реакция амплификации. Детекцию продукта амплификации проводили в 3%-м агарозном геле.*

Результаты. *У больных тяжёлой пневмонией на фоне гриппа A/H1N1 частота развития полиорганной дисфункции (SOFA ≥ 2 баллов) составила 24 (43,6 %) случая. При анализе частоты встречаемости минорного аллеля Gly, согласно генетическим моделям, установлены различия между пациентами 1-й и 2-й групп в кодоминантной (p = 0,023; отношение шансов (ОШ) – 8,82 (0,95–81,89)) и доминантной (p = 0,005; ОШ = 12,35 (1,40–109,07)) моделях.*

Заключение. *Тяжёлое течение пневмонии при гриппе A/H1N1 сопровождается высокой частотой развития органной дисфункции. Риск развития органной недостаточности увеличивался у больных тяжёлой пневмонией с выявленным полиморфизмом гена TLR4 Asp299Gly, что, вероятно, требует дальнейшего изучения.*

Ключевые слова: *TLR4, полиморфизм, грипп A/H1N1, пневмония, органная дисфункция*

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INTRODUCTION

The systemic inflammatory response, being the basis for the formation of critical conditions, regardless of the inducing factor, proceeds in two phases – from a hyperinflammatory reaction to a compensatory anti-inflammatory response [1, 2]. In the pro-inflammatory phase, damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) initiate signaling, activating innate and subsequently adaptive immune responses. To date, various physiological and pathophysiological mechanisms of innate and adaptive immune responses have been identified, implemented through the involvement of a large repertoire of immune receptors in the process, one of which is Toll-like receptors (TLR) [3]. Moreover, single-nucleotide polymorphisms of the Toll receptor genes have been identified, in some cases leading to a change in signal transmission realized through the receptor, which significantly affects the function of the receptor and contributes to the pathogenesis of inflammation and infectious diseases [3].

One of the most well-studied Toll receptors is TLR4. The main ligand of the receptor is a bacterial lipopolysaccharide, but TLR4 is also able to bind endogenous structures, such as heat shock proteins (HSP), including HSP70, Gp96, HSP22 and HSP7, proteins S100A8 and S100A9, as well as extracellular matrix molecules (ECM), such as biglican, tenascin-C, versican, and fragments of molecules ECM, including hyaluronic acid oligosaccharides and heparan sulfate [4]. The *TLR4* gene is located on 9q32-33 chromosome. The polymorphic locus rs4986790 is a single-nucleotide substitution of adenine (A) for guanine (G) at the +896 position of exon 3 (896A>G), leading to an amino acid substitution of aspartic acid for glycine at position 299 of the polypeptide chain of the Asp299Gly receptor [5]. The described *TLR4* gene mutation is associated with the lack of a proper immune response upon receptor activation. The *TLR4* Asp299Gly polymorphism has been shown to be associated with the development of a number of diseases [5–7]; it is of interest to study the role of the *TLR4* Asp299Gly gene polymorphism in critically ill patients with severe pneumonia associated with A/H1N1 influenza.

THE AIM OF THE STUDY

To identify the incidence of the *TLR4* Asp299Gly gene polymorphism and determine its contribution to the development of organ dysfunction in patients with severe pneumonia associated with A/H1N1 influenza.

MATERIALS AND METHODS

The study included 55 patients with severe pneumonia associated with A/H1N1 influenza hospitalized in ICU/CCU of the Municipal Clinical Hospital No. 1 (Chita), the Regional Clinical Hospital (Chita), the Regional Clinical Infectious Diseases Hospital of the Trans-Baikal Territory during the morbidity peak in 2019. The study was conducted in compliance with the principles of the World Medical Association Declaration of Helsinki (1964, ed. 2013), and approved by the local Ethics Committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation (Protocol No. 84 dated March 01, 2017). Inclusion criteria: severe pneumonia; consolidation/ground-glass syndrome according to chest X-ray/CT. Verification of the pathogen in the respiratory swab was carried out using PCR method: A/H1N1 influenza virus RNA was identified. The age of the patients was 47 [38; 62] years. The proportion of male was 47.8 %, female – 52.2 %. Exclusion criteria: unstable hemodynamics; body mass index > 30; diabetes mellitus; HIV; tuberculosis, oncopathology. To diagnose and assess the severity of pneumonia, the CURB/CRB-65, SMART-COP scales, as well as the federal clinical guidelines of the Ministry of Health of the Russian Federation "Community-acquired pneumonia in adults" and the IDSA/ATS criteria (in the presence of one "large" or three "small" criteria, pneumonia was regarded as severe) were used. In order to assess the degree of organ dysfunction, the following were used: qSOFA scale (Sequential Organ Failure Assessment [Quick]) (respiratory rate ≥ 22/min; systolic blood pressure ≤ 100 mmHg; decrease in consciousness < 15 points as per the Glasgow scale) – 1 point for each block; SOFA scale, which included an assessment of consciousness as per the Glasgow Coma Scale in points, a modified respiratory coefficient as the ratio of oximetry as a percentage to the oxygen content in the inhaled air in units (SpO_2/FiO_2), the level of bilirubin and creatinine in blood serum, the number of blood platelets, the level of average blood pressure with the presence or absence of inotropic and (or) vasopressor support in points. The patients were divided into two groups: Group 1 – patients with SOFA score ≥ 2; group 2 – patients with SOFA score < 2. Gene SNPs were determined by PCR method using standard kits developed by Research and Production Company "Litekh" (Moscow). Amplification of *TLR4* gene fragments was carried out in a thermal cycler "Bis-M111" (Bis-N LLC, Novosibirsk). Genomic DNA isolated from whole blood leukocytes using the "DNA Express Blood" reagent was analyzed followed by an amplification reaction. The amplification product was detected in a 3% agarose gel. Statistical processing of the obtained data was carried out using Statistica 10 software package (StatSoft Inc., USA) and SNPStats online calculators (https://medstatistic.ru/calculators.html). The distribution of genotypes was evaluated for compliance with the Hardy – Weinberg equilibrium. The genotype frequencies in the groups were compared using the Yates' continuity-corrected χ^2 test using the contingency table; if the expected phenomena in one of the cells were less than 5, the Fisher's exact test was used. To assess the association of genotypes with disease severity, the odds ratio (OR) was calculated with 95% confidence intervals (95% CI) using five genetic models: codominant, dominant, recessive, superdominant and log-additive.

RESULTS

In patients with severe pneumonia associated with A/H1N1 influenza, the incidence of multiple organ dysfunction (≥ 2 points on the SOFA scale) was 24 (43.6 %) cases, men prevailed among them – 17 (70.8 %) patients. In the structure of concomitant pathology among pneumonia patients with organ dysfunction, the most common were: chronic obstructive pulmonary disease – 7 (29.1 %) cases; type 2 diabetes mellitus – 6 (25.0 %) cases; alcoholism – 4 (16.6 %) cases. Furthermore, 16 (66.6 %) alimentary-constitutional obesity was revealed in patients as a background pathology. In the second patient group, ischemic heart disease was more common – in 9 (29.0 %) cases. Systemic inflammatory response syndrome (SIRS) was detected in 55 (100 %) cases. When analyzing the structure of organ dysfunction according to the SOFA scale, the highest incidence of organ failure development was found in the blocks of oxygenation, coagulation, central nervous system (CNS) function and hemodynamics parameters (Fig. 1). Besides, the frequent combination of organ disorders among patients with severe pneumonia associated with A/H1N1 influenza draws attention: for example, a combination of hemostasis and oxygenation disorders was noted in 10 (41.6 %) cases, and a combination of disorders of oxygenation, hemostasis, hemodynamics and CNS function – in 6 (25 %) cases.

The *TLR4* Asp299Gly gene polymorphism was studied in the test groups (Table 1). The value of $\chi^2 = 7.493$ for two degrees of freedom, with a statistical significance level of $p = 0.024$, which indicates statistically significant differences between comparable groups. When studying the *TLR4 Asp299Gly* gene polymorphism, a statistically significant difference in the groups was found for the Asp/Asp genotype ($p = 0.021$; OR = 0.081; 95% CI: 0.009–0.715). At the same time, there was no statistically significant difference in the incidence of the Asp/Gly variant and the homozygous Gly/Gly variant, which is probably due to the small sample size and low frequency of occurrence of the Gly allele (Table 1).

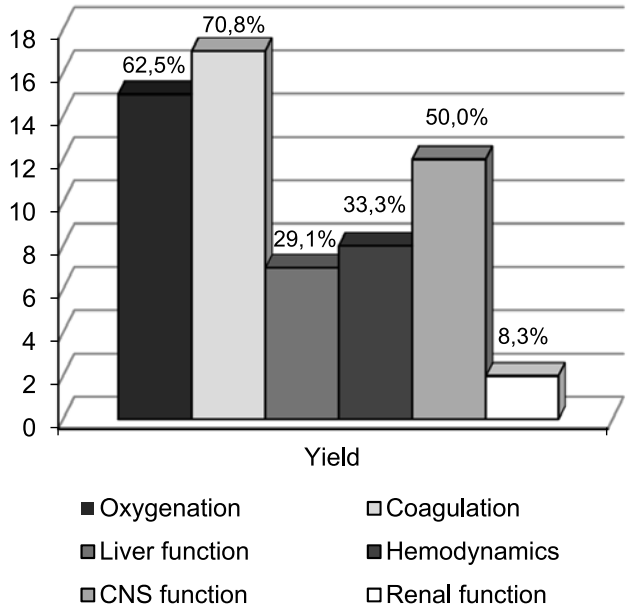

FIG. 1.
The structure of organ dysfunction by the SOFA scale blocks in patients with severe pneumonia associated with A/H1N1 influenza

When analyzing the frequency of occurrence of the minor Gly allele, according to genetic models, the most significant result was established for the dominant model ($p = 0.0052$; OR = 12.35 (95% CI: 1.40–109.07)), according to which the genotype carrying at least one Gly allele has an increased risk (Table 2). The results obtained in the superdominant and log-additive models are considered as uninformative at the obtained genotype distribution frequencies.

DISCUSSION

One of the leading pathophysiological components of a critical condition and organ dysfunction development is systemic inflammation. In the pro-inflammatory phase,

TABLE 1

DISTRIBUTION OF *TLR4* GENE GENOTYPES IN PATIENTS WITH SEVERE PNEUMONIA ASSOCIATED WITH A/H1N1 INFLUENZA

Genotypes	Frequency of genotypes		Fisher's exact test, *p*	*p* Bonferroni adjusted	OR	95% CI
	1st group (*n* = 24)	2nd group (*n* = 31)				
Asp/Asp	17 (70.8 %)	30 (96.8 %)	0.0159	0.0477	0.081	0.009–0.715
Asp/Gly	5 (20.8 %)	1 (3.2 %)	0.0755	0.226	7.895	0.855–72.882
Gly/Gly	2 (8.3 %)	0	0.186	0.558	–	–

TABLE 2

GENETIC MODELS OF THE ASSOCIATION OF *TLR4* GENE GENOTYPES WITH THE RISK OF ORGAN DYSFUNCTION DEVELOPMENT IN PATIENTS WITH SEVERE PNEUMONIA ASSOCIATED WITH A/H1N1 INFLUENZA

Genotypes	1st group (*n* = 24)	2nd group (*n* = 31)	OR (95% CI)	*p*
		Codominant model		
Asp/Asp	17 (70.8 %)	30 (96.8 %)	1.00	
Asp/Gly	5 (20.8 %)	1 (3.2 %)	8.82 (0.95–81.89)	0.02369
Gly/Gly	2 (8.3 %)	0 (0.0 %)	–	
		Dominant model		
Asp/Asp	17 (70.8 %)	30 (96.8 %)	1.00	0.005191
Asp/Gly-Gly/Gly	7 (29.2 %)	1 (3.2 %)	12.35 (1.40–109.07)	
		Recessive model		
Asp/Asp-Asp/Gly	22 (91.7 %)	31 (100 %)	1.00	0.1859
Gly/Gly	2 (8.3 %)	0 (0 %)	2.7 (0.32–153)*	

Note. * – adjusted value for a small sample.

damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) initiate signaling, activating innate and subsequently adaptive immune responses [2, 8]. Detection of conservative molecular patterns associated with pathogens (for example, lipopolysaccharide) through leucine-rich repeats (LRR) leads to dimerization of TLR, bringing together the TIR (Toll-interleukin receptor) signaling domains, forming intracellular docking platforms that allow recruiting adapter proteins and kinases into the signal transmission process, inducing nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and immune response [9, 10]. The single-nucleotide polymorphism of the *TLR4* 896A>G gene is associated with a decrease in the intensity of the immune response to TLR4 stimulation by lipopolysaccharide of gram-negative bacteria [11]. At the same time, as a pattern recognition receptor, TLR4 interacts not only with PAMPs, but also with a number of endogenous structures involved in the systemic inflammatory response cascade, such as heat shock proteins (HSP), including HSP70, Gp96, HSP22 and HSP7, proteins S100A8, and S100A9 [4]. In addition, the intensity of the emerging immune response depends on the ligand interacting with TLR4 [12]. The relationship of the *TLR4* Asp299Gly polymorphism with the course of the infectious process and inflammation in various diseases has been described [6, 7, 13]; a relationship with the severity of the critical condition in sepsis has been also shown [14]. This study demonstrated the prevalence of the Asp299Gly *TLR4* mutant allele and the combination of Arg753Gln *TLR2,* Leu412Phe TLR3 polymorphisms among influenza and influenza pneumonia patients [15]. We obtained comparable results: the presence of the *TLR4* gene polymorphic variant in patients with severe pneumonia associated with A/H1N1 influenza is connected with an increase in the severity of the disease and is accompanied by an increase in the frequency of organ dysfunction in this category of patients.

CONCLUSION

The severe course of pneumonia associated with A/H1N1 influenza is accompanied by a high incidence of organ dysfunction. The revealed presence of the *TLR4* Asp299Gly gene polymorphism was accompanied by an increased risk of organ failure, which probably requires further study.

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Conflict of interest

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

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MORPHOLOGY, PHYSIOLOGY AND PATHOPHYSIOLOGY

CHANGES IN THE REACTIVITY OF THE VERTEBROBASILAR ARTERIES WHEN USING GLUCOSE-ELECTROLYTE DRINK WITH ANTIOXIDANT PLANT EXTRACTS DURING SUBMAXIMAL EXERCISE TEST

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ABSTRACT

The aim. *To assess the effect of glucose-electrolyte composition with plant extracts having antioxidant activity on the hemodynamic parameters of vertebrobasilar system during the incrementally increasing submaximal exercise test.*
Materials and methods. *The study included 12 athletes (6 candidates for master of sports and 6 masters of sports) aged 18–22, who have been engaged in orienteering for 10 years and more. Time of aerobic exercise – 2 hours a day, five days a week. The study subjects performed an incrementally increasing submaximal exercise test and also submaximal exercise test with the preventive intake of a glucose-electrolyte composition with plant extracts having antioxidant properties.*
To assess the hemodynamic parameters in all study subjects we used Doppler ultrasound of the cerebral vessels, evaluating vertebrobasilar system blood flow, exercise gas test in the modification of hypo- and hyperventilation, and also positional test.
Results. *A single intake of glucose-electrolyte drink under conditions of incrementally increasing exercise test contributed to the manifestation of a homeostatic effect in hemodynamic parameters of the vertebrobasilar arteries. It is evidenced by the approximation to the pre-exercise level of maximum systolic velocity and average blood velocity in the breath-holding test, of the diastolic blood velocity in the hyperventilation test, and of the pulsatility index in the torsion test, as compared to the isolated submaximal exercise test which caused the change in both velocity indicators and calculated indices during the functional tests.*
The article considers the main mechanisms underlying the change in arterial hemodynamic parameters caused by incrementally increasing load, as well as describes the proposed mechanisms arising from the combined effect of an incrementally increasing load and the intake of a glucose-electrolyte composition with plant extracts having antioxidant activity.
Conclusion. *It was shown that using glucose-electrolyte drink contributed to the restoration of hemodynamic parameters of the vertebrobasilar arteries after the incrementally increasing submaximal exercise test.*

Key words: *incrementally increasing submaximal exercise test, athletes, glucose-electrolyte drink, vertebrobasilar arteries*

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ИЗМЕНЕНИЕ РЕАКТИВНОСТИ АРТЕРИЙ ВЕРТЕБРОБАЗИЛЯРНОГО БАССЕЙНА ПРИ ПРИЁМЕ ГЛЮКОЗО-ЭЛЕКТРОЛИТНОГО НАПИТКА С ДОБАВЛЕНИЕМ АНТИОКСИДАНТНЫХ РАСТИТЕЛЬНЫХ ЭКСТРАКТОВ ПРИ НАГРУЗОЧНОМ ТЕСТИРОВАНИИ СУБМАКСИМАЛЬНОЙ МОЩНОСТИ

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РЕЗЮМЕ

Цель исследования. *Оценка влияния глюкозо-электролитного состава с добавлением растительных экстрактов, обладающих антиоксидантной активностью, на гемодинамические показатели вертебробазилярного бассейна при выполнении ступенчато-возрастающей нагрузки субмаксимальной мощности.*

Материалы и методы. *В исследовании принимали участие 12 спортсменов (6 кандидатов в мастера спорта и 6 мастеров спорта) в возрасте 18–22 лет, занимающихся спортивным ориентированием в среднем 10 лет и более. Испытуемые выполняли ступенчато-возрастающую нагрузку субмаксимальной мощности, а также выполняли нагрузку субмаксимальной мощности с превентивным приёмом глюкозо-электролитного состава с добавлением растительных экстрактов, обладающих антиоксидантными свойствами. Для оценки гемодинамических показателей всем испытуемым проводили ультразвуковую допплерографию сосудов мозга, оценивая показатели кровотока в вертебробазилярном бассейне, а также нагрузочные газовые пробы – в модификации гипо- и гипервентиляции; кроме того, проводили позиционную пробу.*

Результаты. *Однократный приём глюкозо-электролитного напитка в условиях нагрузки ступенчато-возрастающей мощности способствовал проявлению гомеостатического эффекта в отношении гемодинамических показателей артерий вертебробазилярного бассейна, о чём свидетельствует приближение к уровню донагрузочных значений максимальной систолической скорости и средней скорости кровотока в пробе с задержкой дыхания, диастолической скорости кровотока – в пробе с гипервентиляцией, показателей индекса пульсации – в торсионной пробе, в отличие от изолированного выполнения нагрузки субмаксимальной мощности, после прохождения которой изменялись как скоростные показатели, так и расчётные индексы в ходе проведения функциональных проб.*

Рассмотрены основные механизмы, лежащие в основе изменения гемодинамических показателей артерий под действием ступенчато-возрастающей нагрузки, а также описаны предполагаемые механизмы, возникающие при комбинированном воздействии ступенчато-возрастающей нагрузки и приёме глюкозо-электролитного состава с добавлением растительных экстрактов, обладающих антиоксидантной активностью.

Заключение. *Показано, что приём глюкозо-электролитного напитка способствовал восстановлению гемодинамических показателей артерий вертебробазилярного бассейна после ступенчато-возрастающей нагрузки субмаксимальной мощности.*

Ключевые слова: *нагрузочное тестирование субмаксимальной мощности, спортсмены, глюкозо-электролитный напиток, артерии вертебробазилярного бассейна*

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INTRODUCTION

Functional activity of the brain in humans is largely determined by the continuous delivery of oxygen, metabolic nutrients which are determined by the active regulation of cerebral blood flow. Therefore, maintaining proper blood flow in the brain is the most important task of the cardiovascular system, including during periods of intense physical activity, along with increasing blood flow to functioning muscles. In particular, during exercise, despite a significant increase in cardiac output up to 300–600 % [1] and blood flow in skeletal muscles up to 800 %, there is a moderate increase in blood flow in brain vessels by 10–30 %, which ensures maintenance of proper substrate delivery due to synergetic integration of the nervous, hormonal and humoral systems. According to recent studies, there is a significant heterogeneity in the response of cerebral vessels to increasing exercises and to changes in blood gas composition [2]. Moreover, it was shown that the most evident changes during intensive endurance exercise are noted to a greater extent in the vessels of the vertebrobasilar system, which is manifested as the most significant increase in perfusion in the vertebral arteries in comparison with the arteries of the carotid system [2]. The greatest sensitivity to changes in blood gas composition has also been shown for the vessels of the vertebrobasilar system by positron emission tomography (PET), according to which vasomotor reactions to hypercapnia were higher in the vertebrobasilar circulation zones than in most of the cerebral cortex supplied by the carotid system [3]. Accordingly, impaired hemodynamics of this system is accompanied by significant neurological disorders.

One of the causes of hemodynamic disorders in professional athletes is dehydration and hyponatremia occurring during prolonged (more than 40 minutes) intensive endurance training. If water and sodium requirements remain unmet, encephalopathy may develop due to overheating [4].

In milder cases, fluid volume deficit that occurs during prolonged exercise is a factor accompanying a decrease in performance. At the same time, a significant decrease in performance and endurance begins with a dehydration level of 2 % or higher [5], which is accompanied by a decrease in aerobic metabolism and, as a consequence, a deterioration in energy supply, resulting in a significant decrease in sports performance. Thus, the total loss of fluid and sodium during moderate exercise lasting more than an hour at an optimal temperature is 1 liter or more. Fluid volume deficit initiates the release of vasopressin, activation of the renin-angiotensin-aldosterone system to urgently replenish its losses. A consequence of this is an increase in blood viscosity, hypovolemia, an increase in endothelial dysfunction, an increase in cardiac rate [6].

In the study of A.N. Martinchik et al. [7] published results showing that "bottled water is the main method of rehydration in 86 % of 280 examined athletes (candidates and masters of sports). Moreover, 95–96 % of athletes in the group of martial arts and power sports use water. In other sports, there are fewer water consumers – 67–79 %. As for sports drinks, only 31 % of highly qualified endurance sports athletes and a small percentage of martial arts athletes use them only during training."

In 2018, updated clinical recommendations of the Federal Medical and Biological Agency on methods of rehydration of the athletes' body [7] were published, consistent with the main theses of the European Commission Scientific Committee on Food aimed at compensating daily losses during heavy muscle activity, especially in athletes [8], and their specifications. According to these clinical guidelines, drinks for athletes engaged in endurance sports should contain at least two types of sugar and should also be sodium-enriched – so-called glucose-electrolyte solutions or carbohydrate-electrolyte formulations. The content of the remaining trace elements is non-obligatory [9, 10], however, only in the case of a balanced diet of athletes [11].

It has been proven that during prolonged physical activity, solutions containing optimal sugar concentrations improve performance, increase endurance by supplying working muscles with carbohydrates and preventing depletion of glycogen reserves, maintaining the oxygen balance in the blood with the help of antioxidants [12].

Currently, sports drinks in a wide range are presented mainly by foreign manufacturers. Domestic goods are represented by few items. For nutrient enrichment, endurance growth and training effectiveness, herbal extracts with antioxidant properties are often added to sports drinks, although the effect of the latter on the functioning of muscle tissue under high exercise activity is currently a debatable issue [13]; however, the role of antioxidants for the cardiovascular system, including under sports exercises, has high evidence-based potential [14].

At the same time, given the biodiversity of vegetation, the developed essential oil production in the Republic of Crimea, as well as the developed sports sector (orienteering, sports tourism), it is proposed to improve the basic formulation of glucose-electrolyte composition by adding aqueous extracts of rosehip fruit, nettle leaf, lemon juice, as well as rosemary hydrolate, which have strong antioxidant properties, and enrichment with vitamin and mineral premix that helps nutrient enrich athletes.

Accordingly, **the aim of this study** was to assess the effect of glucose-electrolyte composition with antioxidant plant extracts, based on plant raw materials produced in ecologically clean regions of the Republic of Crimea, on the blood flow indicators of the vertebrobasilar system during the incrementally increasing submaximal exercise test.

MATERIALS AND METHODS

The study was carried out at the Center for the Collective Use of Scientific Equipment "Experimental Physiology and Biophysics" of the V.I. Vernadsky Crimean Federal University.

The study involved 12 athletes (6 candidates for masters of sports and 6 masters of sports) aged 18–22 years en-

gaged in orienteering. The experience of sports exercises averaged 10 years or more. Aerobic exercise mode – 2 hours a day for five days a week.

To assess hemodynamic parameters, all subjects underwent ultrasound dopplerography (USDG) of vessels, assessing blood flow in the vertebrobasilar system (symmetrical vertebral arteries and the basilar artery), as well as gas stress tests – in the modification of hypo- and hyperventilation; in addition, a positional test was performed. USDG of brain vessels was performed on Sonomed 300 (Nizhny Novgorod).

To assess the effect of glucose-electrolyte composition on the hemodynamic parameters of the vertebrobasilar system, two study cycles were conducted:

1. The first cycle of the study included velocity indicators recording, as well as calculated indices of USDG along the arteries of the vertebrobasilar system. In addition, in the first cycle of the study, functional tests were carried out, including hyper- and hypoventilation tests with simultaneous registration of USDG indicators along the basilar artery and positional tests of symmetrical vertebral arteries, also with simultaneous registration of USDG indicators.

During the study, the following indicators of blood flow along the basilar and vertebral arteries (symmetrical) were assessed:

V_{max} – maximum systolic blood flow velocity;

V_{min} – diastolic blood flow velocity;

V_{aver} – average blood flow velocity;

Ri – Pourcelot's resistance index, which reflects the state of resistance to blood flow distal to the measurement site and is the ratio of the difference between the maximum systolic and end diastolic velocities to the maximum systolic velocity:

$$Ri = \frac{(V_{max} - V_{min})}{V_{max}};$$

Pi – pulsatility index (also known as the Gosling index), which reflects the elastic properties of the arteries, decreases with age and represents the ratio of the difference between the maximum systolic and diastolic velocities to the average velocity:

$$Pi = \frac{(V_{max} - V_{min})}{V_{aver}}.$$

Additionally, ventilation tests were performed to assess the state of the metabolic circuit of cerebral blood flow regulation by locating the basilar artery.

Before conducting a breath-holding test, the subject was warned about the need to exhale on signal and hold his/her breath for the maximum possible period. Hemodynamic parameters were recorded during the period of breath holding at the time of a steady change of velocity indicators, after which the subject could inhale and resume the usual breathing rhythm.

The hyperventilation test was started after a complete stable recovery of the USDG pattern. When performing a hyperventilation test, the subject began forced breathing. Monitoring of the hyperventilation tests was started from the moment of intensive breathing, waiting for the moment when the amplitude of the spectral signals would stop changing; the last 5–6 complexes were recorded on the screen, after which the subject resumed the usual breathing rhythm.

After complete restoration of the USDG pattern, a positional (torsion) test was performed along the vertebral arteries on both sides sequentially. During the positional test, the subject performed a slow turn of the head and neck away from the sensor, achieving maximum amplitude (chin to shoulder), lowering his/her head so as to reach the level of the shoulder joint with his/her nose.

After the background (baseline) study of USDG, the subjects were offered 250 ml of bottled water with the addition of a flavoring agent imitating the aroma of the drink under study.

After the USDG and intake of bottled water, the athletes were offered an exercise test, including an incrementally increasing load on the Kettler bicycle ergometer (KETTLER Holding GmbH, Germany) from the starting position of 50 Watts followed by an increase by 50 Watts every 3 min of the exercise stage. The exercise test was completed at the request of the athlete – "up to failure".

As soon as the athletes performed the submaximal exercise test, USDG values of the vertebrobasilar arteries were recorded again, taking into account the performance of functional tests.

2. The second cycle was carried out two weeks after the first one. The second cycle was methodically similar to the first one, with the difference that in the second cycle, as a replenishment of fluid and electrolytes, athletes were offered a glucose-electrolyte composition with the addition of plant extracts with antioxidant properties in the amount of 250 ml 15 min before the exercise test. The athletes were stopped at the same exercise stage at which they completed the exercise in the first cycle.

Glucose-electrolyte solution composition includes a natural mineral complex in the form of sea salt of the Black Sea obtained by natural evaporation in the waters of Sasyk-Sivash Lake – 0.4 g; prepared drinking water – up to the required volume, rosemary hydrolate (aromatic water) – 65 ml; water extract of rosehip fruits (1:10) – 100 ml; water extract of nettle leaf (1:10) – 100 ml; maltodextrin, glucose, lemon juice – 5 ml; ascorbic acid, potassium sorbate, sodium benzoate. Glucose-electrolyte drink complies with TR CU 022/2011, standardized according to GOST R 56543-2015 (Table 1).

The subjects who took part in the study did not have any chronic cardiovascular or respiratory pathology. None of the athletes took drugs that affect vascular tone. On the day of the study, the subjects did not undergo any preliminary physical activities. The study was conducted in the morning (10^{00}–13^{00}).

All procedures performed in human studies comply with the ethical standards of the National Committee for Ethics in Research and the Declaration of Helsinki (1964) and its subsequent revisions or comparable

ethical standards. Informed voluntary consent was obtained from each of the participants included in the study. Minutes of the meeting of the Ethics Committee of V.I. Vernadsky Crimean Federal University No. 1 dated January 25, 2022.

TABLE 1

ENERGY VALUE AND CHEMICOANALYTICAL COMPOSITION OF THE DRINK

Indicator	Content	
	in 100 ml	in 250 ml
Carbohydrates (g), incl.	8.0	20.0
glucose, g	2.0	5.0
maltodextrin, g	6.0	15.0
Ascorbic acid, g	0.2	0.5
Sodium, mg	37.6	94.0
Calcium, mg	1.6	4.0
Potassium, mg	21.2	53.0
Chlorides, mg	84.0	210.0
Essential oil, g	0.016	0.04
Energy value, kcal/kJ	23/97	58/241

The Shapiro – Wilk test was used to check the distribution of the data array for normality, and the results showed that the data array did not follow a normal distribution. In this regard, the Friedman non-parametric test was used to determine the statistical significance of differences in the linked sample when comparing three arrays. In the case of pairwise comparison of linked samples, the Wilcoxon test was used. For clarity of description and visualization of the results obtained, the average values were used, as well as the error of the mean.

Calculations and graphic design of the data obtained in this paper were carried out using Microsoft Excel (Microsoft Corp., USA) and Statistica 8.0 software package (StatSoft Inc., USA).

STUDY RESULTS

As the results of the first and second study cycles showed, the main differences in the USDG indicators, recorded after the incrementally increasing submaximal exercise test, were only available in relation to the data recorded during the functional tests in comparison with the level of pre-exercise values of the corresponding indicators.

The values of hemodynamic parameters of the vertebrobasilar arteries registered outside the functional tests after the incrementally increasing submaximal exercise test were close to the baseline values (Table 2). Such stability of blood flow velocity indicators indicates a high degree of fitness and endurance during aerobic exercises and, as a result, a high degree of synchronization and stability of both the cardiorespiratory system and the musculoskeletal system during incrementally increasing aerobic exercise.

The results of the first study cycle, when athletes performed only an incrementally increasing submaximal exercise test, indicate changes in a number of velocity indicators and calculated indices, recorded during functional tests after incrementally increasing load, in comparison with the level of pre-exercise data of the corresponding indicators, recorded during similar tests (Table 3).

The results of the second study cycle, when athletes took a glucose-electrolyte drink followed by submaximal exercise, indicate the stability of the hemodynamic parameters of the investigated arteries, since the values of the studied indicators obtained after exercises and taking a glucose-electrolyte drink were close to the baseline values (Table 3).

In particular, the results of the first cycle study indicate that the performance of the incrementally increasing submaximal exercise test is accompanied by an increase in the V_{max} index by 21.07 % ($p < 0.05$) during the period of the breath-holding test in the basilar artery relative to the data of this indicator registered in the pre-exercise period during a similar test.

The results of the second cycle study indicate that performing submaximal exercise test and taking a glucose-electrolyte drink prevent changes in the values of the V_{max} index in comparison with the level of pre-exercise values of this indicator in the breath-holding test.

A comparative analysis of the results of the first and second study cycles indicates that the V_{max} index was lower by 14.57 % ($p < 0.05$) in the case of glucose-electrolyte drink intake and exercise relative to the data of this indicator recorded during isolated exercise test during the breath-holding test, and approached the level of pre-exercise values.

Thus, glucose-electrolyte drink intake prevented changes in the V_{max} index when performing submaximal exercise test during the breath-holding test.

Similarly, the dynamics of the V_{aver} indicator changed during the breath-holding test. Thus, the performance of incrementally increasing exercise test in the first cycle was accompanied by an increase in this indicator by 24.58 %

TABLE 2

DYNAMICS OF PARAMETERS OF DOPPLER ULTRASOUND OF VERTEBROBASILAR ARTERIES IN PROFESSIONAL ORIENTEERS, REGISTERED BEFORE AND AFTER INCREMENTALLY INCREASING LOAD, AS WELL AS BEFORE AND AFTER INCREMENTALLY INCREASING LOAD IN COMBINATION WITH TAKING A GLUCOSE-ELECTROLYTE DRINK

Study phases	The arteries under study	USDG indicators of vertebrobasilar arteries				
		V_{max}, cm/s	V_{min}, cm/s	V_{aver}, cm/s	Ri	Pi
Background (cycle I)	BA	70.09 ± 4.06	32.74 ± 1.38	42.15 ± 1.66	0.53 ± 0.01	0.88 ± 0.06
	VA on the right	57.62 ± 2.73	27.09 ± 2.57	36.26 ± 2.19	0.52 ± 0.04	1.04 ± 0.28
	VA on the left	51.78 ± 6.79	30.73 ± 4.88	38.03 ± 5.39	0.45 ± 0.03	0.68 ± 0.08
Exercise (cycle I)	BA	73.56 ± 6.96	35.7 ± 3.63	46.8 ± 4.32	0.51 ± 0.02	0.83 ± 0.10
	VA on the right	52.8 ± 5.86	30 ± 3.17	35.8 ± 5.87	0.43 ± 0.21	0.67 ± 0.10
	VA on the left	52.8 ± 5.97	27.2 ± 5.83	35.6 ± 6.13	0.48 ± 0.08	0.77 ± 0.21
Background (cycle II)	BA	70.16 ± 3.78	33.10 ± 1.18	43.78 ± 2.85	0.52 ± 0.02	0.89 ± 0.06
	VA on the right	53.02 ± 4.01	28.80 ± 2.01	37.05 ± 2.95	0.53 ± 0.03	1.01 ± 0.07
	VA on the left	52.50 ± 3.28	30.54 ± 2.59	36.15 ± 2.14	0.48 ± 0.02	0.65 ± 0.04
Glucose-electrolyte composition + exercise (cycle II)	BA	65.6 ± 3.97	30.35 ± 1.8	37.95 ± 1.27	0.53 ± 0.03	0.92 ± 0.08
	VA on the right	67.51 ± 5.32	33.34 ± 3.54	40.78 ± 3.21	0.48 ± 0.03	0.81 ± 0.11
	VA on the left	50.45 ± 4.27	27.67 ± 2.27	32.65 ± 2.09	0.44 ± 0.03	0.69 ± 0.07

Note. BA – basilar artery; VA – vertebral artery.

($p < 0.05$) relative to the data of this indicator recorded before submaximal exercise (Fig. 1).

The intake of glucose-electrolyte composition and subsequent incrementally increasing exercise test did not significantly change the values of this indicator in comparison with the level of pre-exercise values recorded in the breath-holding test.

A comparative analysis of the results of cycles I and II of the study shows a decrease in the V_{aver} index by 18.37 % ($p < 0.05$) after submaximal exercise test in combination with the intake of glucose-electrolyte composition in comparison with the data of this indicator obtained after the isolated submaximal exercise test, and an approximation of V_{aver} index values in cycle II to the level of pre-exercise values obtained during the breath-holding test.

Thus, taking a glucose-electrolyte drink prevented changes in V_{max} and V_{aver} indices after the submaximal exercise test, and the results of the incrementally increasing exercise test after the tested drink intake are comparable with baseline data recorded in the pre-exercise period during the breath-holding test.

V_{min} had similar dynamics of change as the V_{max} and V_{aver} indices, but without achieving a statistically significant difference.

During the hyperventilation test, there were also changes in velocity indicators along the basilar artery.

The results of the hyperventilation test showed similar, but not identical results.

Thus, V_{min} increased by 25.87 % ($p < 0.05$) after the isolated incrementally increasing exercise test relative to base-

TABLE 3

DYNAMICS OF PARAMETERS OF DOPPLER ULTRASOUND OF VERTEBROBASILAR ARTERIES OF PROFESSIONAL ORIENTEERS DURING FUNCTIONAL TESTS REGISTERED BEFORE AND AFTER INCREMENTALLY INCREASING LOAD (CYCLE I), AS WELL AS BEFORE AND AFTER INCREMENTALLY INCREASING LOAD IN COMBINATION WITH TAKING A GLUCOSE-ELECTROLYTE DRINK

Study phases	The arteries under study	USDG indicators of vertebrobasilar arteries				
		V_{max}, cm/s	V_{min}, cm/s	V_{aver}, cm/s	Ri	Pi
Background (cycle I)	BA (hyperventilation)	61.25 ± 3.05	28.92 ± 1.54	36.71 ± 1.83	0.52 ± 0.01	0.87 ± 0.05
	BA (breath holding)	64.39 ± 3.27	32.17 ± 1.93	40.79 ± 2.28	0.49 ± 0.02	0.80 ± 0.07
	VA (torsion) on the right	50.50 ± 3.74	24.77 ± 1.13	33.48 ± 1.41	0.49 ± 0.02	0.74 ± 0.05
	VA (torsion) on the left	43.42 ± 2.9	25.26 ± 1.43	28.03 ± 1.61	0.51 ± 0.04	0.80 ± 0.07
Exercise (cycle I)	BA (hyperventilation)	73.93 ± 1.61 $p < 0.05$	35.61 ± 2.56 $p < 0.05$	43.90 ± 3.19	0.51 ± 0.04	0.90 ± 0.15
	BA (breath holding)	77.96 ± 7.81 $p < 0.05$	40.82 ± 5.36	50.82 ± 4.83 $p < 0.05$	0.47 ± 0.04	0.75 ± 0.13
	VA (torsion) on the right	49.06 ± 7.01	28.53 ± 3.40	36.8 ± 4.29	0.41 ± 0.01	0.54 ± 0.03 $p < 0.05$
	VA (torsion) on the left	39 ± 10.42	20 ± 2.01	19.8 ± 3.32	0.42 ± 0.11	0.45 ± 0.14 $p < 0.05$
Background (cycle II)	BA (hyperventilation)	61.38 ± 3.48	28.36 ± 1.28	37.01 ± 1.45	0.53 ± 0.02	0.87 ± 0.04
	BA (breath holding)	64.24 ± 2.47	31.79 ± 1.32	40.21 ± 2.16	0.46 ± 0.03	0.81 ± 0.04
	VA (torsion) on the right	51.29 ± 2.99	24.17 ± 1.73	34.67 ± 1.31	0.48 ± 0.01	0.75 ± 0.04
	VA (torsion) on the left	44.12 ± 2.91	25.71 ± 1.92	27.05 ± 1.72	0.53 ± 0.03	0.80 ± 0.06
Glucose-electrolyte drink + exercise (cycle II)	BA (hyperventilation)	72.8 ± 6.35 $p < 0.05$	30.62 ± 2.88 $p_1 < 0.05$	37.82 ± 2.41 $p_1 < 0.05$	0.56 ± 0.03	1.12 ± 0.14 $p < 0.05$
	BA (breath holding)	66.60 ± 3.65 $p_1<0.05$	33.77 ± 1.99	41.48 ± 1.44 $p < 0.05$	0.49 ± 0.01	0.79 ± 0.04
	VA (torsion) on the right	55.61 ± 4.10	27.24 ± 1.87	34.12 ± 1.97	0.50 ± 0.03	0.83 ± 0.11 $p_1 < 0.05$
	VA (torsion) on the left	54.8 ± 6.01	29.52 ± 3.95	32.41 ± 4.35	0.46 ± 0.03	0.83 ± 0.1 $p_1 < 0.051$

Note. $p < 0.05$ is statistical significance of the differences according to the Wilcoxon test when comparing baseline values and values obtained during exercise test in cycle I, and statistical significance of the differences obtained when comparing baseline values and values obtained after taking glucose-electrolyte drink followed by an incrementally increasing load in cycle II; $p_1 < 0.05$ is statistical significance of the differences according to the Friedman test when comparing background values and values obtained after performing isolated exercise test and exercise in combination with glucose-electrolyte drink.

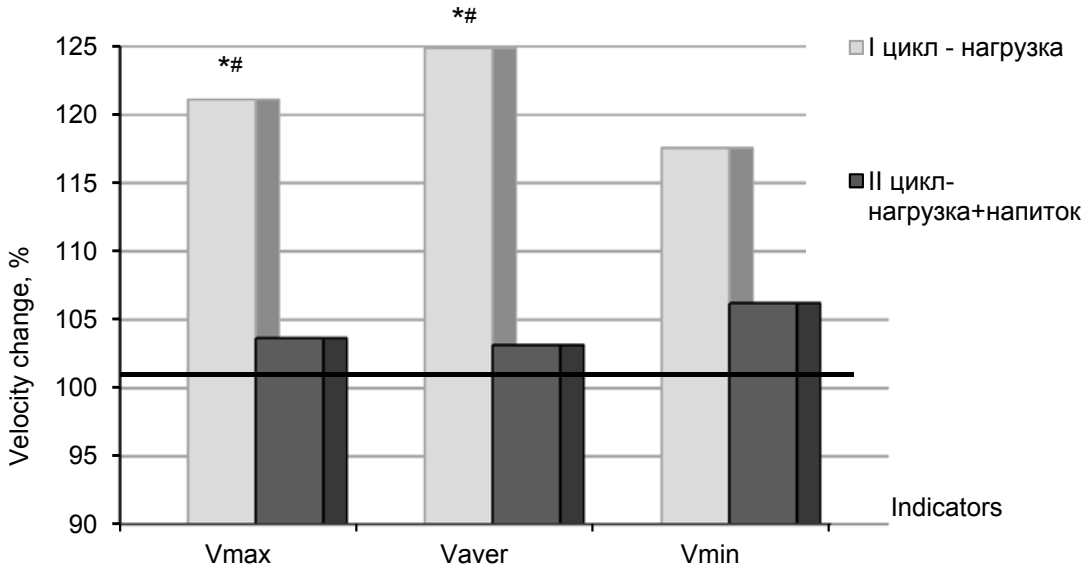

FIG. 1.

*The changes in the velocity indicators of the basilar artery during breath-holding test before and after an incrementally increasing load (cycle I), as well as before and after an intake of the glucose-electrolyte drink followed by an incrementally increasing load (cycle II), in percent relative to the values recorded before the load (taken as 100 %): * – statistical significance of differences according to the Wilcoxon between background values and values obtained during exercise test in the cycle I, and between background values and values obtained after the combination of an intake of the glucose-electrolyte drink with exercise in the cycle II ($p < 0.05$); # – statistical significance of differences according to the Friedman test between background values and values obtained after an isolated exercise test and after the combination of an intake of the glucose-electrolyte drink with exercise ($p_1 < 0.05$)*

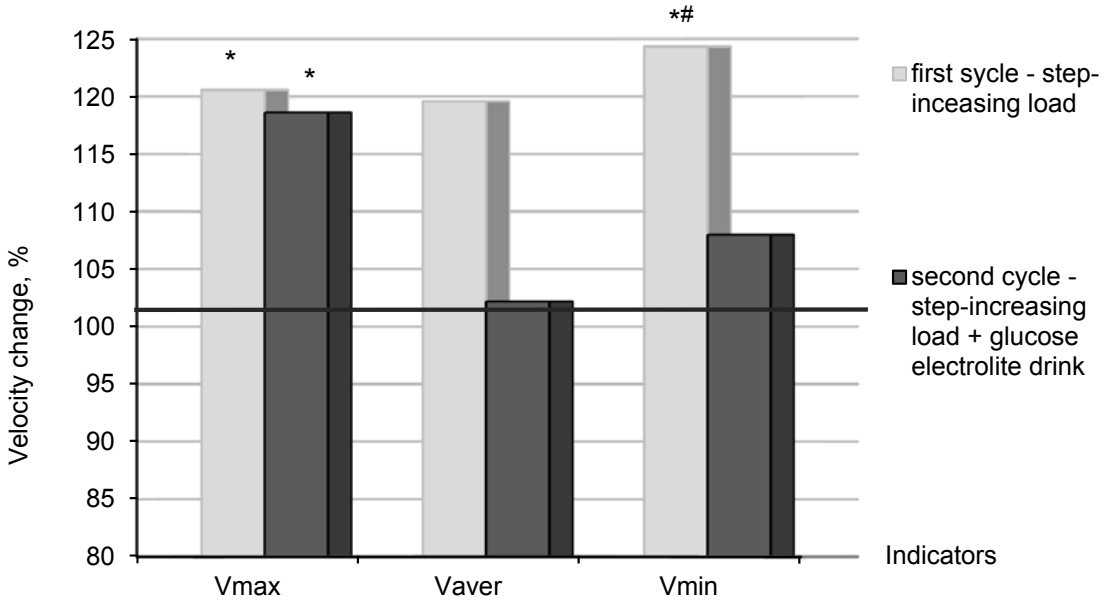

FIG. 2.

*The changes in the velocity indicators of the basilar artery during hyperventilation test before and after an incrementally increasing load (cycle I), as well as before and after an intake of the glucose-electrolyte drink followed by an incrementally increasing load (cycle II), in percent relative to the values recorded before the load (taken as 100 %): * – statistical significance of differences according to the Wilcoxon between background values and values obtained during exercise test in the cycle I, and between background values and values obtained after the combination of an intake of the glucose-electrolyte drink with exercise in the cycle II ($p < 0.05$); # – statistical significance of differences according to the Friedman test between background values and values obtained after an isolated exercise test and after the combination of an intake of the glucose-electrolyte drink with exercise ($p_1 < 0.05$)*

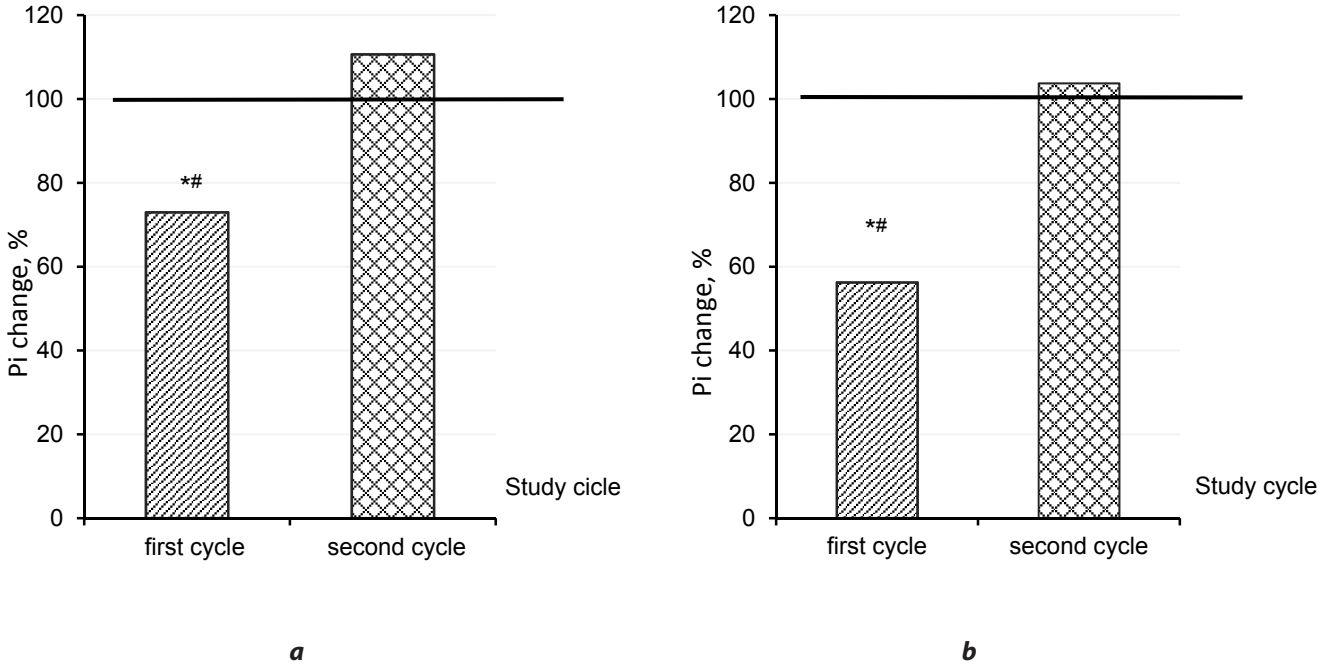

FIG. 3.

*The changes in the Gosling index (Pi) on the vertebral artery right (a) and left (b) during the torsion test before and after an incrementally increasing load (cycle I), as well as before and after an intake of the glucose-electrolyte drink followed by an incrementally increasing load (cycle II), in percent relative to the values recorded before the load (taken as 100 %): * – statistical significance of differences according to the Wilcoxon between background values and values obtained during exercise test in the cycle I, and between background values and values obtained after the combination of an intake of the glucose-electrolyte drink with exercise in the cycle II ($p < 0.05$); # – statistical significance of differences according to the Friedman test between background values and values obtained after an isolated exercise test and after the combination of an intake of the glucose-electrolyte drink with exercise ($p_1 < 0.05$)*

line data of this indicator recorded during the hyperventilation test (Fig. 2).

Glucose-electrolyte drink intake and subsequent incrementally increasing exercise test did not statistically significantly change the V_{min} value during the hyperventilation test.

A comparative analysis of the results of the first and second study cycles shows a 14.01 % ($p < 0.05$) decrease of V_{min} index in cycle II after exercise and drink intake in comparison with the values of this indicator recorded in cycle I after an isolated exercise test during the hyperventilation test (Fig. 2). Thus, the intake of the drink under study followed by exercise prevented changes in the V_{min} index in the hyperventilation test.

A slightly different dynamics characterized the V_{max} index during the hyperventilation test. Thus, V_{max} increased after isolated exercise test by 19.32 % ($p < 0.05$) relative to baseline data for this indicator.

Glucose-electrolyte drink intake and subsequent incrementally increasing exercise test were also accompanied by an increase in this indicator by 18.61 % ($p < 0.05$) in comparison with its baseline data recorded during the hyperventilation test (Fig. 2).

There was no difference in V_{max} during the hyperventilation test after exercise in cycles I and II.

Consequently, glucose-electrolyte drink intake did not prevent the change in V_{max} during the hyperventilation test.

During the positional (torsion) test assessing extravasal influences, stability in relation to most velocity indicators to the athletes' performance of a submaximal exercise test was noted along the vertebral arteries. Changes after an isolated exercise test and exercise in combination with taking a glucose-electrolyte drink were noted only according to the Gosling index data.

Thus, when registering a torsion test along the vertebral artery on the right side, performing incrementally increasing exercise test led to a one-third decrease in the Gosling index ($p < 0.05$) relative to the values of this indicator recorded in the pre-exercise period during the torsion test.

Taking glucose-electrolyte drink in combination with submaximal exercise test contributed to the inversion of the response – a tendency to increase this indicator to values of 0.84 ($p < 0.05$) (Fig. 3a), comparable with its baseline values.

Differences in Pi registered after performing an isolated exercise test and performing exercise in combination with taking glucose-electrolyte composition reached values of 53 % ($p < 0.05$). It is worth noting that the values of this indicator registered in cycle II were close to the pre-exercise level in comparison with the values obtained in cycle I of the study (Fig. 3a).

Similar dynamics of the Gosling index were recorded during a torsion test along the vertebral artery on the left

side. Isolated exercise test contributed to a 43.75 % ($p < 0.05$) decrease in Pi in comparison with the pre-exercise period during the test (see Fig. 3b).

The values of the Gosling index after taking a glucose-electrolyte drink and performing submaximal exercise test practically were practically unchanged in cycle II of the study and corresponded to the level of pre-exercise values.

The resulting differences in the Gosling index values after exercise for cycles I and II of the study were over 50 % ($p < 0.05$) (see Fig. 3b).

Thus, a single intake of a glucose-electrolyte drink followed by exercise contributed to the manifestation of a homeostatic effect with respect to the mechanisms of regulation of arterial hemodynamics in the vertebrobasilar system under incrementally increasing submaximal exercise test conditions, as evidenced by the stability of most hemodynamic parameters recorded after taking the drink and performing incrementally increasing exercise test, as opposed to an isolated submaximal exercise test after passing which both velocity parameters and calculated indices changed during functional tests.

RESULTS DISCUSSION

During the period of intense physical activity, taking into account the high energy consumption of the brain and the absence of significant intracellular energy reserves, accurate control of the intake of nutrients and by-products is supported by changes in cerebral circulation, which is coordinated by the operation of various regulatory mechanisms. If the mechanisms of regulation of vascular tone at rest are well known, then during the period of active physical activity they have not yet been fully determined. Basal vascular tone, vegetative innervation and regional heterogeneity in ion channels or NO production play a significant role. However, the greatest contribution to the regulation of cerebral blood flow during aerobic exercise is made by changes in arterial blood gas content, metabolic changes, arterial pressure, cardiac output, neurovascular innervation, as well as endothelial reactivity [15]. The separation of these contributions is highly problematic due to the redundancy of the cardiovascular system to achieve complex regulation, which has led to discrepancies in the definition of key participants in the regulation of blood flow during exercise [15].

As it was shown in this study, submaximal exercise and subsequent breath-holding test were accompanied by a marked increase in both systolic and average velocity values in comparison with the data of the corresponding indicators recorded before performing submaximal exercise in the course of a similar test.

The increase in velocity indicators during the breath-holding test is a response to an increase in partial pressure of CO_2, which is accompanied by an increase in the H^+ level, resulting in an increase in the osmolarity level and the subsequent dilation reaction. However, performing a submaximal exercise followed by a breath-holding test is accompanied by a large decrease in pH due to lactate accumulation, which may be accompanied by changes in cerebral hemodynamics and an increase in blood flow, on the one hand. This was probably the reason for the increase in velocity indicators along the basilar artery after submaximal exercise in the period of the breath-holding test in comparison with the data during a similar test before submaximal exercise.

On the other hand, blood flow is linearly dependent on cardiac output, which increases in proportion to exercise [16]. At the same time, contrary to the recently prevailing views about the constancy of cerebral hemodynamics under any conditions, including when performing physical activity, according to the studies of recent years, the response of cerebral blood flow to prolonged aerobic exercise is described as biphasic, with a distinct progressive increase, respectively increasing exercise intensity to approximately 60 % of maximum consumption oxygen with subsequent access to the plateau [15]. Probably, the increase in V_{max}, depending on cardiac output, after submaximal exercise during the breath-holding test can be explained both by an increase in cardiac output and a more intense decrease in the pH level.

Hyperventilation test after submaximal exercise was accompanied by an increase in the V_{min} index, which indicates a decrease in peripheral resistance of resistive vessels and probably dictated by metabolic changes, namely a moderate drop in pH due to lactate accumulation relative to the data of this indicator recorded before the athletes performed exercises during a similar test.

In addition, as per the results of the torsion test, after the athletes performed submaximal exercise test, there was a decrease in the Gosling index, depending on the elasticity of the vascular wall. Arterial elasticity, in turn, is determined by both structural elements and tone of vascular smooth muscles. And if the first group of factors is more stable, then the second depends largely on the level of functional activity of the vascular endothelium due to the significant representation of receptors to a huge number of humoral factors on its surface and the ability to release both constrictors and dilators in response.

There are studies indicating endothelial dysfunction in professional athletes engaged in endurance sports [17].

The supposed mechanism of endothelial dysfunction in athletes lies in the avalanche-like production of reactive oxygen species that oxidize low-density lipoprotein molecules with the formation of oxidized low-density lipoprotein (oxLDL) [18], whose high content in the bloodstream is toxic to endotheliocytes, since oxLDL can induce necrosis and apoptosis of endothelial cells. In this case, oxLDL lead to activation of immunocompetent cells accompanied by autosensitization and accumulation of autoantibodies in blood. Antibodies, forming immune complexes with oxLDL, have an additional damaging effect on the endothelium. In this regard, the endothelium reactionary activity, which is actively involved in adaptive reactions to physical activity, changes. And if, normally, the balance of functional activity of the endothelium shifts towards the release of vasodilators, anti-inflammatory, and an-

tithrombic factors, then when the endothelium is attacked by immune complexes with oxLDL – towards the release of vasoconstrictors, as well as prothrombic and proinflammatory factors. This is supported by experimental data that among skiers, athletes engaged in rowing, as well as cycling, signs of early atherosclerosis and a tendency to thrombosis were detected in 60.5 % of cases [19]. A study by Z.V. Lopatin and V.S. Vasilenko [20] showed an increase in the level of homocysteine up to 18 mmol/L and D-dimer in professional athletes engaged in endurance sports. Moreover, hyperhomocysteinemia is determined at the plasma concentration of 10 μmol/L and higher and is associated with the development of cardiovascular diseases due to endothelial dysfunction development and contributes to an increase in the risk of acute cerebral circulation disorders of both ischemic and hemorrhagic types due to promotion of atherogenesis and atherothrombosis of cerebral vessels, increased activity of matrix metalloproteinases [21].

At the same time, oxidative stress caused by homocysteinemia significantly reduces the bioavailability of nitric oxide and leads to the dysregulation of intracellular signaling and gene expression.

During intensive athletes' training, there is an increase in systolic pressure, which entails constant changes in shear stress and stimulates the production of not only nitric oxide, but also free oxygen forms. In the chronic mode, such changes cause endothelial overstimulation effect, as well as maladaptation [22].

It is possible to assume that a glucose-electrolyte drink with antioxidant plant extracts behaves similarly to the adaptogens of natural origin, although it does not belong to the classical representatives of this kind, since the leading components in the composition of the drink under study are rosemary hydrolate, lemon juice, nettle leaf, rosehip fruit vitamin premix, as well as trace elements. Traditionally, natural adaptogens are ginseng, rhodiola rosea, *Schisandra chinensis* (lemongrass), Eleutherococcus, leuzea, and arabia. It is these adaptogens that have a large evidence base, including in relation to sports achievements [13]. A number of substances that make up the plant extracts (on the basis of which the drink under study was made) are also part of traditional adaptogens, probably in lower concentrations, and in combination with other substances. Probably, the tested glucose-electrolyte composition with antioxidant plant extracts affects various links of regulatory mechanisms that control hemodynamics of the studied vessels.

Thus, during the incrementally increasing submaximal exercise test, it is natural to overload the electron transport chain in the mitochondrial membranes, which results in the production of an excessive number of free radicals that damage mitochondrial membranes, which leads to a decrease in cell energy. In this respect, a single intake of a glucose-electrolyte drink with antioxidant plant extracts, including lemon juice, as well as nettle and rosehip extract, due to which high ascorbic acid content is achieved, has an effect on changing energy processes in cells. In addition, the composition of rosehip and traditional adaptogens, such as rhodiola rosea and Schisandra chinensis (lemongrass), combine organic acids, represented mainly by citric, succinic and malic. The content of organic acids, especially citric and succinic in combination with ascorbic acid significantly intensifies the activity of the Krebs cycle. Besides, ascorbic acid, being involved in the biochemical processes of inhibiting free-radical reactions, probably contributes to a decrease in the activity of lipid peroxidation processes [23].

Rosemary hydrolate containing rosmarinic acid helps tissues to better utilize glucose [24]. In a study by M. Prasannarong et al. [25] it was shown that even a single dose of rosmarinic acid at a dose of 10 mg/kg helped to reduce angiotensin II effects and improved glucose transport to the skeletal muscles.

The fructose solution, which is part of the glucose-electrolyte composition, is a donor of the nutrient substrate.

In addition, nettle extract, as has been shown in recent studies, in combination with endurance exercise improves the functioning of mitochondria, as well as the expression of nuclear respiratory factor 2 of the heart muscle and gamma coactivator 1-alpha [26].

Accordingly, the intake of the investigated drink containing fructose solution in combination with rosmarinic acid, lemon juice, nettle and rosehip extracts forms a chain of reactions, on the one hand, supplying the substrate, ensuring its high utilization and effective oxidation, and on the other hand, the intake of glucose-electrolyte composition shifts the pH level to the alkaline side. It is likely that a moderate increase in pH after taking the glucose-electrolyte composition, as well as activation of energy processes, contributed to the restoration of autoregulation, which probably stabilized the V_{max} and V_{aver} velocity indicators during the breath-holding test after submaximal exercise test (see Fig. 1).

A single consumption of the studied glucose-electrolyte drink by athletes and the subsequent performance of the incrementally increasing submaximal exercise test also helped to prevent the increase in V_{min} observed after the isolated exercise during the hyperventilation test. The stabilization of this indicator after taking the tested drink and exercise indicates restoration of arterial tone, probably due to the effect of flavonoids such as rutin, quercetin, heperedin, toning the vascular wall, which are part of rosehip in the studied drink. The same substances are part of traditional adaptogens, such as *Rhodiola rosea* and *Leuzea*.

Taking a glucose-electrolyte drink did not affect the dynamics of V_{max} in the hyperventilation test after the first and second cycles. The absence of other distinct changes in professional athletes during the hyperventilation test within the first and second cycles of the study may indicate that oxygenation appears to be a fairly strong regulatory factor [16], in comparison with other metabolic and humoral factors.

In addition, the intake of glucose-electrolyte composition contributed to the stabilization of the Gosling index (pulsatility index) after submaximal exercise, whereas isolated exercise performance contributed to its sig-

nificant drop. Given the dependence of this indicator on the elasticity of the vascular wall, which in turn is mostly determined by vascular endothelium functional activity, it can be assumed that the effect of taking the glucose-electrolyte drink was induced by substances in its composition, in particular, by polyphenolic compounds contained in the lemon juice, rosemary hydrolate, and rosehip extract.

It is known that flavonoids contained in rosehips, such as hesperidin, quercetin, rutin, have high antioxidant, anti-inflammatory activity, and also have a high tropism to the vascular system [27], in particular to the vascular endothelium, changing its apoptosis pathways [28], promoting nitric oxide increase, improving its responsiveness to influencing factors by modulating endothelium-dependent vasodilation [29]. Intake of products containing polyphenolic compounds, in particular bioflavonoids, affects the following mechanisms of endothelium-dependent vasodilation: an increase in the level of Ca^{2+} and redox activation of the phosphoinositide-3 (PI3) kinase/Akt pathway are noted in endotheliocytes, leading to rapid and sustained activation of nitric oxide synthase (NOS) [30], as well as the formation of an endothelium-dependent hyperpolarization factor. In addition to the described long-term effects of flavonoids, short-term effects are also known, including NO-mediated vasorelaxation due to an increase in the influx of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+} in endothelial cells. This is supported by evidence that an increase in endothelium-dependent vasodilation is observed even after a single intake of ascorbic acid [30].

CONCLUSION

Thus, the isolated performance of the incrementally increasing submaximal exercise test contributes to changes in velocity indicators and calculated indices of the vertebrobasilar arteries during functional tests performance with breath-holding, hyperventilation, as well as a torsion test, which indicates the development of tension regulation of cerebral blood flow in the conditions of the studied load regime. A single intake of glucose-electrolyte composition with antioxidant plant extracts contributes to stabilization of hemodynamic parameters of the vertebrobasilar arteries under incrementally increasing submaximal exercise test conditions, preventing changes in parameters observed after isolated exercise. Thus, taking the studied drink with antioxidant properties under incrementally increasing submaximal exercise test conditions contributed to the stabilization of the maximum and average velocity indicators in the breath-holding test, approaching to the background pre-exercise values of the minimum velocity indicator in the hyperventilation test, as well as to the "return" to the pre-exercise values of the pulsatility index in the torsion test from both sides.

The studied glucose-electrolyte composition has a homeostatic effect, probably due to the effect on the mechanisms of homeostatic regulation of vascular tone, affecting endothelial reactivity, pH level stabilization, oxidative phosphorylation.

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Conflict of interest

The authors declare no conflict of interest.

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NEUROLOGY AND NEUROSURGERY

THE EFFECT OF ACUPUNCTURE ON THE CORRECTION OF POST-STROKE DYSPHAGIA COMPARED TO METHODS OF PHYSICAL INFLUENCE

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ABSTRACT

Background. *Dysphagia is a problematic field in the clinical work of neurologists, resuscitation experts and therapists due to the high percentage of secondary complications caused by aspiration pneumonia, nutritional deficiency, which affect the recovery process. This ultimately affects the main socio-medical indicators such as mortality, lethality, disability in structure of cerebrovascular diseases.*
Neurogenic dysphagia occurs in 25–65 % of patients with stroke, while mortality among patients with post-stroke dysphagia receiving tube feeding varies from 20 to 24 %.
The aim. *To evaluate the impact of reflexology in the treatment of post-stroke dysphagia in comparison with the methods of physical impact.*
Materials and methods. *An open clinical comparative study was conducted in two clinical bases: Republican Hospital named after N.A. Semashko (Ulan-Ude) and Bokhan District Hospital. In this clinical trial, 53 patients with swallowing disorders during the acute period of ischemic stroke were tested. When diagnosing dysphagia, a point scale of the Clinic of the Institute of the Brain was used, which assesses the degree of swallowing disorders before and after treatment. The comparative group receiving standard therapy in combination with physiotherapy (VOCASTIM) included 27 patients, the study group was comprised of 26 patients, who underwent basic therapy in combination with acupuncture (1 course – 10 sessions). In the course of the comparative clinical trial, statistical data were obtained that indicate the positive role of acupuncture in restoring the function of swallowing in patients with acute impairment of cerebral circulation.*
Results. *During the current clinical study, on the background of a 10-day course of reflexology with an exposure of 10–15 minutes and VOCASTIM physiotherapy for 10–15 minutes, the majority of patients experienced regression of post-stroke dysphagia.*
Conclusion. *According to the results of this study, the method of reflexology as a result of physiotherapy showed a faster recovery of swallowing function in the correction of neurogenic swallowing diseases, which causes cerebral infarction.*

Key words: *ischemic stroke, rehabilitation, reflexology, dysphagia, physiotherapy*

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ВЛИЯНИЕ АКУПУНКТУРЫ НА КОРРЕКЦИЮ ПОСТИНСУЛЬТНОЙ ДИСФАГИИ В СРАВНЕНИИ С МЕТОДАМИ ФИЗИЧЕСКОГО ВОЗДЕЙСТВИЯ

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РЕЗЮМЕ

Введение. *Дисфагия является проблемным полем в клинической работе неврологов, реаниматологов и терапевтов ввиду высокого процента вторичных осложнений, вызванных аспирационной пневмонией, нутритивным дефицитом, влияющих на восстановительный процесс, что в итоге влияет на основные социально-медицинские показатели, такие как смертность, летальность, инвалидизация в структуре цереброваскулярных заболеваний. Нейрогенная дисфагия встречается у 25–65 % больных с инсультом, при этом летальность среди пациентов с постинсультной дисфагией, получающих зондовое питание, варьирует от 20 до 24 %.*

Цель. *Оценить влияние рефлексотерапии в лечении постинсультной дисфагии в сравнении с методами физического воздействия.*

Материалы и методы. *Открытое клиническое сравнительное исследование проводилось на двух клинических базах: ГАУЗ «Республиканская клиническая больница имени Н.А. Семашко» МЗ Республики Бурятия и ОГБУЗ «Боханская районная больница». В данном клиническом исследовании апробированы 54 пациента, имеющие нарушения функции глотания в остром периоде ишемического инсульта. При диагностике дисфагии использовалась балльная шкала, разработанная ООО «Клиника института мозга», оценивающая степень нарушения функции глотания до и после лечения. В сравнительную группу, получающую стандартную терапию в сочетании с физиотерапией (VOCASTIM), вошло 27 больных; 27 пациентам, составившим исследуемую группу, проводилась базисная терапия в сочетании с иглорефлексотерапией (1 курс – 10 сеансов).*

Результаты. *В ходе нашего сравнительного клинического исследования на фоне проведённого 10-дневного курса рефлексотерапии с экспозицией 10–15 минут и физиотерапией VOCASTIM 10–15 минут, у большинства пациентов отмечается регресс постинсультной дисфагии.*

Заключение. *По результатам данного исследования, метод рефлексотерапии в сравнении с физиотерапией показал более эффективное восстановление функции глотания в коррекции нейрогенных глотательных нарушений, причиной которых является инфаркт мозга.*

Ключевые слова: *ишемический инсульт, реабилитация, рефлексотерапия, дисфагия, физиотерапия*

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INTRODUCTION

Stroke remains the most important medical and social problem, both in the world and in Russia, which is caused by high rates of morbidity, mortality and disability [1]. According to statistical analysis, stroke affects about 0.5 million people annually in Russia with an incidence rate of 3 per 1000 of the population [2].

The high level of disability is primarily due to the severity of neurological disorders which are difficult to correct. Dysphagia occupies the most significant place among neurological disorders, the cause being infarction of the stem structures and basal ganglia. Despite the achievements of modern medicine, dysphagia most often gives secondary complications in the form of aspiration pneumonia, reduces the patient's quality of life and requires constant monitoring by medical staff and relatives.

Swallowing is a multiphase motor process, which is realised through voluntary and involuntary (reflex) movements of the head and neck muscles, with three phases of the swallowing act: oral, pharyngeal and esophageal. The process of swallowing is carried out by 26 muscles with nerve regulation from six pairs of cranial nerves: V, VII pairs of cranial nerves with nuclear localisation at the level of the pons varolii and brain stem, IX–XII pairs with nuclear localisation at the level of the medulla oblongata and spinal cord I–V cervical segments.

Dysphagia is manifested by impaired passage of food from the mouth to the stomach and may be either neurogenic or mechanical, e. g. as a result of a neoplasm in the oesophagus. [3]. Depending on the phase of swallowing, oropharyngeal and oesophageal dysphagia are classified. The most frequent type of dysphagia is oropharyngeal, with an incidence of up to 81 %, as a consequence of damage to the centers regulating the swallowing act [4].

Dysphagia is quite common not only among older people in need of nursing care and ICU patients, but also among independent older people in their everyday environment. Mortality in patients with neurogenic dysphagia treated with tube feeding can be as high as 24 % [5]. According to one of the leading rehabilitation centers of the Clinical Institute of Brain LLC (Berezovsky, Russia), dysphagia occurs in almost every second patient who has suffered a traumatic brain injury, and in every fourth – after a stroke [6].

Neurogenic (motor) dysphagia in acute cerebrovascular accident (ACVA) is caused by ischaemic lesions of the cerebral cortex, basal ganglia, brain stem or cerebellum.

Neurogenic dysphagia in combination with motor disorders, for example, hemiparesis, requires increased attention and careful diagnosis [7, 8].

Originating in ancient China, reflexology has a deep history and successfully proves its effectiveness in the treatment of neurological diseases. The relevance of this topic arises from the low level of research in national and European medicine, where little published material is available.

In Russian medicine, there is evidence of a positive effect of both medicinal methods of treatment (cholinergic drugs – neuromidin, axamon) and physiotherapeutic methods (VOCASTIM) [9]. In turn, foreign scientific research has data on clinical studies conducted on the treatment of dysphagia by acupuncture. L. Chen et al. and W. Xia et al. studied the effect of acupuncture on the restoration of swallowing function in two clinical studies. The authors conducted the study in the acute period and early recovery period after stroke. The treatment period was 7 weeks in the first study and 4 weeks in the second, where there was positive evidence of efficacy of acupuncture compared to standard care methods [10, 11]. Based on available domestic and international studies, the aim of our study was to conduct a comparative analysis of traditional and unconventional methods of dysphagia treatment.

OBJECTIVE OF THE STUDY

To conduct a clinical study on the effectiveness of acupuncture in the correction of dysphagia in patients with acute ischemic stroke in comparison with physiotherapy.

THE AIM OF THE STUDY:

- to evaluate the effect of corporeal acupuncture on the treatment of dysphagia among patients in the acute period of ischaemic stroke;
- to analyze the effectiveness of physiotherapy in restoring swallowing function among patients with acute cerebrovascular accident;
- to compare the effects between acupuncture and physiotherapy in terms of the recovery process of the swallowing act.

MATERIALS AND METHODS

An open clinical comparative study was conducted in 2021 in two clinical centers: Republican Clinical Hospital named after N.A. Semashko of the Ministry of Health of the Republic of Buryatia and the Bokhan District Hospital. A total of 54 patients were followed up: 27 patients in the study group and 27 in the control group. The patients' ages were 46–78, with a mean age of 63. The numbers of males and females in both groups were comparable. In the majority of patients, the focus of ischaemic stroke was localised to the cortical and stem structures and was confirmed by neuroimaging, using 16-slice MSCT. In 79 % of cases, the stroke was verified in the middle cerebral artery circulation and in 21 % of cases in the areas supplied by the vertebrobasilar system.

All patients underwent a neurological examination according to the generally accepted method. In order to assess the degree of swallowing impairment, a point

scale developed by the Clinical Institute of Brain LLC (CIB) was used. Dysphagia was assessed by a medical speech therapist before and after an acupuncture session. The results were evaluated as follows: 0–2 score – absence of dysphagia, the diet is not limited; 3–7 score – mild dysphagia, a diet with positioning; 8–9 score – moderate dysphagia, a mild diet with restriction; 10–15 score – severe dysphagia, probe feeding is required, fibrolaryngoscopy, training feeding is required.

In the 1st control group (n = 27), patients with swallowing disorders received basic therapy in combination with muscle myostimulation with a VOCASTIM medical complex using plate electrodes. The place of impact is the projection of thyroid cartilage and vocal cords. Myostimulation was carried out in two stages: the first, preparatory stage, was performed with pulsed galvanic current at a frequency of 8 Hz; the second stage, stimulation was performed with monophasic triangular pulse current at a base frequency of 2.5 kHz for a duration of 5–7 minutes. The rate of the pulse current was adjusted individually according to each patient's individual sensations and before the act of swallowing occurred. The duration of stimulation was 10–15 minutes. The course therapy with the VOCASTIM device lasted for 10 days. Swallowing function was assessed using the CIB scale by a speech therapist before the first session and after the 10th session on the 14th day.

In the 2nd study group (n = 27), acupuncture therapy (APT) was administered in combination with baseline therapy for a 10-day session. The APT procedure was administered using 5 cm long disposable stainless-steel needles with a diameter of 0.25 mm. The depth of penetration of the needle is from 0.5 to 1 cun. The duration of the session is 10–15 minutes. The needle insertion procedure was carried out while the patient was lying on the couch in a stationary position. A requirement for the selection of patients in the main and control groups was that the patients were unconscious and without marked cognitive impairment or psychomotor agitation; patients with a high risk of lethal outcome were excluded from the study.

The type of study is a randomized open controlled study. The study was approved by the local Ethics Committee of the Irkutsk State Medical Academy of Postgraduate Education – Branch Campus of the Russian Medical Academy of Continuing Professional Education (Protocol No. 11 dated November 9, 2021). All patients signed a voluntary informed consent form.

Inclusion criteria: diagnosed ischaemic stroke of atherothrombotic genesis; age no older than 78 years; NIHSS score of 20 or less; no depression of consciousness (soporus, coma 1–3).

Exclusion criteria: malignant neoplasms, benign neoplasms located locally in the neck, pharynx, oesophagus; fever of undetermined significance; active tuberculosis; decompensated cardiopulmonary disease; mental illness; nutritional deficiency stage 1–3.

Statistical analysis was performed using Wilcoxon's test for two dependent samples, the pre-treatment and post-treatment scale. The difference in treatment outcomes between the two groups was assessed using the Mann – Whitney U-test.

RESULTS

As part of a comparative study conducted over 10–15 minutes of reflexology and 10–15 minutes of VOCASTIM physiotherapy, the majority of the patients showed a regression of symptoms such as gagging while eating, pain while swallowing, heartburn, salivation, impaired phonation, "wet" voice and malnutrition.

When assessing the swallowing function assessed on the CIB scale, dysphagia was observed in 54 patients, of which in group 1, 27 patients receiving VOCASTIM therapy had mainly an average degree of swallowing dysfunction, the average score on the CIB scale was 7.1; in group 2 (27 patients), mainly moderate degree of swallowing disorder, the average score was 7.2. The dynamics of the re-screening of the CIB swallowing score has been carried out on the 14th day of treatment. The re-assessment of swallowing function after 14 days in the baseline treatment group with VOCASTIM physiotherapy yielded an average score of 5.5. In the study group, after completing the 10th session of acupuncture therapy, the mean values were 4.1 (Fig. 1).

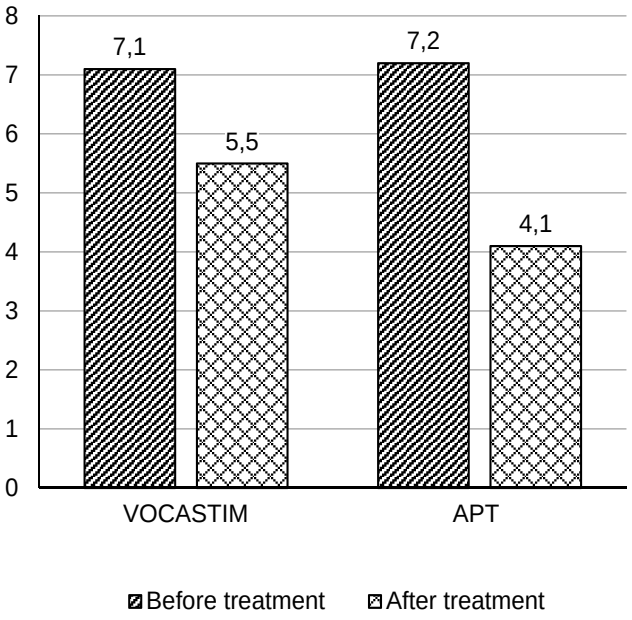

FIG. 1.
Mean CIB scale test value before and after treatment

Against the background of a course of physiotherapy with VOCASTIM device and acupuncture treatment we observed statistically significant effectiveness in restoring swallowing function – Wilcoxon Z-test – 4.1 and 4.5 in groups 1 and 2 respectively (Table 1).

TABLE 1

THE RESULTS OF A NON-PARAMETRIC ANALYSIS OF SHIFTS IN CIB SCALE VALUE BEFORE AND AFTER REHABILITATION TREATMENT IN THE STUDY GROUPS

Groups	Wilcoxon Z-test	*p*
Group 1 (basic + VOCASTIM)	4.1	< 0.001
Group 2 (basic + acupuncture)	4.5	< 0.0001

TABLE 2

THE RESULTS OF NON-PARAMETRIC ANALYSIS OF DIFFERENCES IN THE DISTRIBUTION OF SCALE VALUES BETWEEN GROUPS 1 AND 2 ACCORDING TO THE MANN – WHITNEY TEST

	Median (baseline + acupuncture)	Median (basic + VOCASTIM)	U-test	Z-test	*p*
Dysphagia at discharge	4	6	182	3.157	0.0015

The results of the differences in the distribution of values between groups of patients with the same baseline therapy approach demonstrate that acupuncture recovery rates are statistically significantly higher than those associated with physiotherapy (Table 2).

DISCUSSION

The results of our clinical study obtained among patients with impaired swallowing function caused by cerebral infarction showed positive findings when treated with the acupuncture technique. When comparing the results with those of studies by L. Chen et al. and W. Xia et al. in 2016 [10, 11], there was a positive effect of a longer treatment period of 4–7 weeks compared to our data, where the total course of treatment was 2 weeks of therapy. Most likely, the findings are the result of the different formulations used by the acupuncturist when choosing the treatment of the active biological points.

The positive effect of VOCASTIM's device electrical current is most likely due to the increased flow of afferent impulses from the muscles of the pharynx and the activation of neuroplasticity processes. The therapeutic mechanism of neuromuscular electro-stimulation is probably related to the principle of motor learning, based on repeated repetition of the act of swallowing. During the neuromuscular therapy sessions, the patient made up to 150 swallowing movements per session of electrostimulation, which had an overall positive effect on the tone of the submental muscle groups and consequently the swallowing act in general, as also confirmed by this study.

The role of acupuncture in restoring swallowing function is relevant both because of the high prevalence of dysphagia among post-stroke disorders as well as the positive effects according to the clinical studies conducted.

CONCLUSION

A neurological disorder after a stroke, such as dysphagia, has a difficult course of recovery. This clinical study examines alternative ways to restore swallowing function using acupuncture, as well as physical exposure.

Two groups with predominantly moderate impaired swallowing caused by ischaemic stroke were tested in this study. A comparative analysis was carried out between two groups with different methods of dysphagia treatment – physiotherapy (VOCASTIM device) and acupuncture techniques.

The results of the treatment showed positive changes in both groups, which proves the statistically significant effectiveness of physical and acupuncture treatment in restoring swallowing function.

It is worth noting that according to the results of thi study, the method of reflexotherapy in comparison with physiotherapy, in our opinion, showed a more effective recovery, which is probably associated primarily with a systemic reflexive effect on the neuromuscular system involved in the act of swallowing.

According to the findings, the role of reflexology in restoring swallowing function is statistically significant in effectiveness and can be used in the correction of neurogenic swallowing disorders caused by brain infarc-

tion, thereby affecting the improvement of the rehabilitation process.

Conflict of interest

The authors declare the absence of a conflict of interest.

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THE ROLE OF SPINE ADIPOSE INDEX IN PREDICTING THE RISK FOR SEPTIC SPONDYLODISCITIS AFTER LUMBAR PERCUTANEOUS LASER DISC DECOMPRESSION

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ABSTRACT

The aim. *To analyze the role of the spine adipose index (SAI) in predicting the risk of septic spondylodiscitis after lumbar percutaneous laser disk decompression (PLDD).*
Material and methods*. A retrospective observational single-center study was performed. Various clinical and instrumental parameters have been studied, including the spine adipose index, which are potential risk factors for the development of post-procedural septic spondylodiscitis.*
Results. *The study included 219 patients who underwent PLDD for degenerative lumbar disk disease. The mean post-operative follow-up period for the respondents was 30.8 ± 13.3 months. Signs of septic spondylodiscitis were detected in 5 (2.28 %) cases. Multivariate analysis showed that III degree of anesthesiological risk by the American Society of Anesthesiologists (ASA) scale (p = 0.021), a high value of body mass index (more than 25 kg/m^2) (p = 0.043) and a high value of SAI (over 0.7) (p = 0.037) are statistically significantly associated with the development of septic spondylodiscitis in patients who underwent lumbar PLDD.*
Conclusion. *The value of SAI is statistically significantly associated with the development of spondylodiscitis in patients who underwent PLDD for degenerative lumbar disk disease.*

Key words: *septic spondylodiscitis, spine adipose index, risk factors, percutaneous laser disk decompression*

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РОЛЬ СПИНАЛЬНОГО ЖИРОВОГО ИНДЕКСА В ПРОГНОЗИРОВАНИИ РИСКА РАЗВИТИЯ СЕПТИЧЕСКОГО СПОНДИЛОДИСЦИТА ПОСЛЕ ВЫПОЛНЕНИЯ ЧРЕСКОЖНОЙ ЛАЗЕРНОЙ ДЕКОМПРЕССИИ ПОЯСНИЧНЫХ МЕЖПОЗВОНКОВЫХ ДИСКОВ

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РЕЗЮМЕ

Цель исследования. *Изучить роль спинального жирового индекса (СЖИ) в прогнозировании риска развития септического спондилодисцита после выполнения чрескожной лазерной декомпрессии (ЧЛДД) поясничных межпозвонковых дисков.*

Материал и методы. *Выполнено ретроспективное наблюдательное одноцентровое исследование. Изучены различные клинико-инструментальные параметры, в том числе спинальный жировой индекс, потенциально являющиеся факторами риска развития постпроцедурного септического спондилодисцита.*

Результаты. *В исследование включено 219 пациентов, которым выполнена процедура ЧЛДД по поводу дегенеративного заболевания поясничных межпозвонковых дисков. Средний период послеоперационного наблюдения за респондентами составил 30,8 ± 13,3 месяца. Признаки септического спондилодисцита выявлены в 5 (2,28 %) случаях. Многофакторный анализ показал, что III степень операционно-анестезиологического риска по шкале Американского общества анестезиологов (ASA, American Society of Anaesthesiologists) (p = 0,021), высокое значение индекса массы тела (более 25 кг/м^2) (p = 0,043), а также большое значение СЖИ (свыше 0,7) (p = 0,037) статистически значимо связаны с развитием септического спондилодисцита у пациентов, перенёсших процедуру ЧЛДД на поясничном уровне.*

Заключение. *Значение СЖИ статистически значимо связано с развитием спондилодисцита у пациентов, перенёсших процедуру ЧЛДД по поводу дегенеративного заболевания поясничных межпозвонковых дисков.*

Ключевые слова: *септический спондилодисцит, спинальный жировой индекс, факторы риска, чрескожная лазерная декомпрессия межпозвонковых дисков*

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INTRODUCTION

Septic spondylodiscitis is the most common type of vertebral column infection, affecting the intervertebral cartilage with its adjoining endplates and vertebral bodies [1, 2]. The incidence of spondylodiscitis varies widely and ranges from 0.2 to 2.4 cases per 100,000 of population annually [3]. A number of authors claim that the frequency of occurrence of purulent forms of spondylodiscitis has increased significantly in the last two decades [4–6]. In their opinion, the reasons for such an increase in the prevalence of purulent discitis are: an increase in the average life expectancy of the population, a high incidence of various immunodeficiency conditions, uncontrolled use of antibacterial drugs, as well as an imminent increase in the percentage of performing diagnostic manipulations [5, 6].

The treatment and prevention of septic spondylodiscitis after surgical procedures on the lumbar spine represents a promising area of modern spinal surgery and orthopaedics. Thus, the development of bacterial spondylodiscitis in the early postoperative period is associated with a high incidence of adverse clinical outcomes, an increase in the time of hospitalization of patients and, as a consequence, with an increase in the economic costs of practical healthcare [7]. For this reason, the search for new methods that can predict and prevent the development of septic spondylodiscitis after surgical procedures on the lumbar spine is an extremely urgent problem.

We are aware of a study conducted by V.K. Gupta et al. [8] devoted to the study of the role of the spinal adipose index (SAI) when predicting the risk of development of deep surgical infections after posterior rigid lumbar stabilisation surgery. SAI is the ratio of the thickness of subcutaneous fatty tissue in the lumbar area to the distance between the skin and the vertebral arch [8] (Fig. 1). The authors of this study have clearly demonstrated that SAI plays an important role in predicting the risk of the development of deep surgical site infections among patients who have undergone posterior lumbar fixation surgery.

A literature search of national and international scientific databases revealed a lack of reports devoted to the study of the role of SAI in predicting the risk of septic spondylodiscitis after lumbar percutaneous laser disc decompression (PLDD), which induced the present study.

THE AIM OF THE STUDY

To study the role of spinal fat index in predicting the risk of septic spondylodiscitis after lumbar percutaneous laser disk decompression.

MATERIAL AND METHODS

Study design

A retrospective observational single-centre study was conducted in accordance with international guidelines that govern the conduct and reporting of observational studies in Epidemiology (STROBE, The Strengthening the Reporting of Observational Studies in Epidemiology) [9].

Eligibility criteria

The study included the medical records of patients who had undergone a lumbar PLDD procedure for a degenerative disease of the latter.

The inclusion criteria for the study were:

1) the age of respondents is between 18 and 70;
2) the duration of pain in the lower part of the back and/or lower limbs is at least 6 months;
3) patients' quality of life as measured by the Oswestry Disability Index (ODI) is less than 30 %;
4) ineffectiveness of ongoing conservative treatment for at least 4–6 weeks.

The exclusion criteria for medical records from the study covered:

1) a previous medical history of spinal surgical procedures;
2) a severe neurological impairment;
3) spodylolysis or degenerative spondylolisthesis;
4) central spinal canal stenosis;
5) pregnancy;
6) the history of chronic infectious foci in the body;
7) the failure to provide a full range of clinical and instrumental data from the respondent study;
8) the loss of communication with respondents in the postoperative period.

Proper environment

The study was performed on the basis of the Center for Minimally Invasive Surgery of the Kharlampievskaya Clinic (Irkutsk).

Duration of the study

The study was conducted between March 2021 and April 2022.

Medical intervention description

The lumbar spine LBDD procedure was performed according to generally accepted procedures, with the patient lying on his stomach with lumbosacral flexion, under intravenous sedation and fluoroscopic monitoring by means of a Philips BV Pulsera C-arm (Royal Philips Electronics, The Netherlands). The skin and subcutaneous adipose tissue were tightly infiltrated with a local anaesthetic solution, followed by paraphorominal insertion of a Complete Chiba biopsy needle (Sterylab, Italy), 18 G in diameter and 20 cm long, towards the centre of the nucleus pulposus of the intervertebral cartilage. The next stage was the discography using the X-ray contrast agent Omnipack (GE Healthcare, Ireland) in order to identify structural changes in the fibrous ring. Discography was followed by laser decompression of the intervertebral cartilage using a Mediola Compact diode laser device (Mediola, Belarus). The parameters of the laser radiation were as fol-

lows: wavelength 960 nm, power 7 W, pulse duration 0.6 s, pause duration 1 s, absorbed energy value 1500 J.

Prophylaxis to prevent surgical site infections was performed according to clinical guidelines [10] and included intravenous administration of cefazolin at a dose of 1 g.

The procedure was performed by three neurosurgeons in one operating theatre with the same instrumental equipment.

Study outcomes

The following clinical and instrumental parameters that could potentially be risk factors for septic spondylodiscitis after lumbosacral PLDD were assessed in the study group of patients: gender; age; level of surgical and anaesthetic risk as measured by the American Society of Anaesthesiologists (ASA) scale; body mass index (BMI) value; smoking; diabetes mellitus; number of intervertebral cartilages being operated on; duration of surgical intervention; amount of blood loss; thickness of subcutaneous fatty tissue; and SAI value.

Outcome recording methods

The diagnosis of septic spondylodiscitis has been made according to the clinical, instrumental and laboratory studies, as recommended by the clinical guidelines of the Ministry of Health of the Russian Federation [10]. Subcutaneous adipose tissue thickness and TJI values were calculated on standard T1- and T2-weighted images in sagittal mode using Radiant DICOM Viewer program (Medixant, Poland) using the method of V.K. Gupta et al. [8] (Fig. 1).

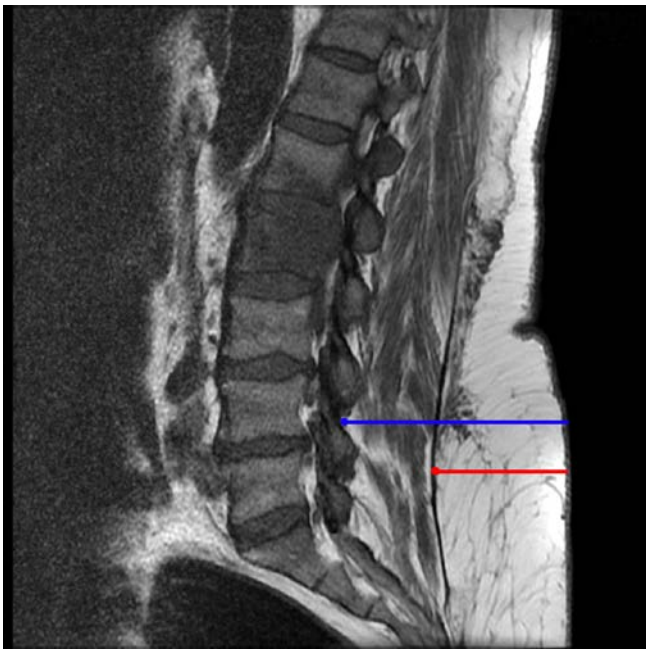

FIG. 1.

An example of the calculation of SAI using the method of V.K. Gupta et al. [8] based on a T2-weighted image of the lumbar spine with signs of septic spondylodiscitis at L_{III}–L_{IV} in the sagittal plane after PLDD

Ethical review

The study protocol was approved by the Ethics Committee of the Irkutsk State Medical University of the Ministry of Health of Russia. The study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki [11].

Statistical data analysis

Continuous variables are represented as mean values (M) and their standard deviations (SD), categorical variables are represented as their number (*n*) and frequency of occurrence (%). A single-factor analysis was performed with the intention of identifying an unadjusted association between the analysed patient parameters and the risk of poor clinical outcomes. The intergroup comparison of categorical variables was performed using Fisher's exact test, continuous variables – using Student's t-test. The collinearity of the covariates was analyzed using the Pearson correlation coefficient. Covariates with a statistically significant effect in a single factor analysis are included in a binary logistic regression model used to identify reliable risk factors for septic spondylodiscitis in patients who underwent a lumbar LBDD procedure. An overall assessment for the consistency of the model and the data obtained was assessed using the Hosmer – Lemeshow test. Statistical data processing was carried out using software Microsoft Excel 2016 (Microsoft Corp., USA) and SPSS 22.0 (IBM Corp., USA). The statistical significance threshold *p* was chosen to be 0.05.

RESULTS

Study participants

According to the eligibility criteria, the present study included 219 patients who had undergone a PLDD procedure for degenerative lumbar intervertebral cartilage disease. The mean post-operative follow-up period for the respondents was 30.8 ± 13.3 months. Signs of septic spondylodiscitis were diagnosed among 5 (2.28 %) patients. In 4 cases, the causative agent was *Staphylococcus aureus* and in 1 case, the etiological agent was *Escherichia coli*. The general characteristics of the patients included in this study are presented in Table 1.

Univariate data analysis

An univariate analysis showing the effect of different parameters on the risk of infective spondylodiscitis among patients with degenerative lumbar intervertebral cartilage disease after PLDD was performed demonstrate the following results. A high degree of surgical and anesthetic risk on the ASA scale (grade III) ($p = 0.029$), the presence of diabetes mellitus ($p = 0.037$), a high BMI value (more than 25 kg/m^2) ($p = 0.024$), as well as a significant subcutaneous adipose tissue thickness (more than 40 mm) ($p = 0.032$) and a high value of SAI (over 0.7) ($p = 0.025$) were associated with the development of spondylodiscitis in the study group of respondents (Table 2).

TABLE 1
GENERAL CHARACTERISTICS OF THE RESPONDENTS INCLUDED IN THE CLINICAL STUDY

Parameters	*n*	%
Gender: male/female	115/104	52.5/47.5
Age (M ± SD)	53.7 ± 8.1	–
Smoking: yes/no	107/112	48.8/51.2
Presence of diabetes mellitus: yes/no	36/183	16.4/83.6
Body mass index value, kg/m^2 (M ± SD)	25.3 ± 12.1	–
Surgical and anaesthetic risk according to the ASA scale		
I degree	177	80.8
II degree	31	14.1
III degree	11	5.1
Number of operated intervertebral cartilages		
1	166	75.7
2	44	20
≥ 3	9	4.3
Duration of surgical intervention, min (M ± SD)	21.4 ± 14.8	–
Volume of blood loss, ml (M ± SD)	11.5 ± 4.2	–
Subcutaneous adipose tissue thickness, mm (M ± SD)	39.7 ± 16.6	–
SAI value (M ± SD)	0.5 ± 0.3	–

Results of multivariate data analysis

A univariate analysis was used to construct a binary logistic regression model including parameters that were statistically significantly associated with the development of infective spondylodiscitis in the patient group under study (Table 3).

According to the constructed model, the III degree of surgical and anesthesiological risk on the ASA scale ($p = 0.021$), a high BMI value (more than 25 kg/m^2) ($p = 0.043$), as well as a high value of SAI (over 0.7) ($p = 0.037$) were statistically significantly associated with the development of septic spondylodiscitis in patients, who underwent the PLDD procedure for a degenerative intervertebral cartilage disease of the lumbar spine. Hosmer – Lemeshow test demonstrated a high degree of consistency between the constructed model and the data obtained ($\chi^2 = 4{,}118$; $p = 0.576$).

DISCUSSION

The conducted study clearly demonstrated that a high value of SAI is a statistically significant risk factor

TABLE 2
RESULTS OF UNIVARIATE ANALYSIS

Parameters	*p*
Male	0.61
Female	0.74
Age (60 years and older)	0.51
Smoking	0.23
The presence of diabetes mellitus	0.037
Body mass index value (more than 25 kg/m^2)	0.024
Surgical and anaesthetic risk according to the ASA scale	
I degree	0.26
II degree	0.18
III degree	0.029
Number of operated intervertebral cartilages	
1	0.74
2	0.48
≥ 3	0.35
Duration of surgical intervention (more than 20 minutes)	0.85
Volume of blood loss (more than 15 ml)	0.97
Subcutaneous adipose tissue thickness (over 40 mm)	0.032
SAI value (over 0.7)	0.025

TABLE 3
RESULTS OF BUILDING A BINARY LOGISTIC REGRESSION MODEL

Parameters	OR (95% CI)	*p*
Smoking	1.69 (0.65–4.31)	0.75
The presence of diabetes mellitus	1.18 (0.33–3.53)	0.42
Body mass index value (more than 25 kg/m^2)	0.81 (0.28–2.29)	0.043
Surgical and anaesthetic risk according to the ASA scale (III)	0.97 (0.96–1.17)	0.021
Subcutaneous adipose tissue thickness (over 40 mm)	0.85 (0.67–1.12)	0.54
SAI value (over 0.7)	0.99 (0.92–1.06)	0.038

Note. OR – odds ratio; 95% CI – 95% confidence interval.

for the development of septic spondylodiscitis after performing lumbosacral PLDD. A literature search of the PubMed, Medline, EMBASE, Cochrane Library and eLibrary scientific databases revealed several reports supporting the effect of SAI values on the risk of surgical infection among patients undergoing posterior stiffening lumbar surgery [12], anterior fixation cervical discectomy [13], and knee and hip endoprosthetics [14]. No similar studies were found regarding the risk of developing dyscitis after the PLDD procedure.

Other studied risk factors for the development of septic spondylodiscitis among patients who have undergone PLDD include a high degree of surgical and anesthetic risk on the ASA scale and a high BMI value. Similar results have been obtained in the clinical series A. Marquez-Lara et al. [15], J.J. Lee et al. [12], as well as in the study of A.O. Mehta et al. [16]. However, these studies also examined groups of respondents who had undergone open decompression-stabilisation surgery on the cervical and lumbar spine. Authors of the aforementioned studies believe that patients with high BMI values and marked subcutaneous fatty tissue thickness are at high risk of surgical infections, including deep localization [15, 16]. The reason for this is the peculiarities of performing surgical access and creating the necessary manipulative tissue corridor to the structures of the spine. Generally, a wide skin incision is then performed in this group of patients, followed by a prolonged insertion of a wound dilator. All this can lead to the contamination of the surgical wound with skin microflora, and a retractor installed causes long-term ischaemia of the tissue mass with the formation of necrosis foci, which subsequently only increases the activity of the infection process [17].

The pathogenesis of septic spondylodiscitis after PLDD is closely related to several factors. For example, inadequate antiseptic preparation of the skin prior to the lumbar PLDD procedure stimulates direct entry of the infective agent into the intervertebral cartilage and, in patients with thick subcutaneous fatty tissue, further spread of micro-organisms and consequently generalisation of the inflammatory process [18]. Haematogenous spread from primary sources of acute or chronic infection also appears to be one of the most important ways for micro-organisms to enter the tissue of the intervertebral cartilage. Blood supply disturbances to the endplates with reduced diffusion of nutrients into the fibrous ring and nucleus pulposus inevitably lead to impaired immune surveillance and the immediate development of an infection process when bacteria and/or fungi penetrate the intervertebral cartilage [19].

Study limitations

Obviously, this study is not free from shortcomings. Firstly, the study is retrospective in nature and includes a small number of patients, which could not but affect the results of statistical analysis of the data obtained. Secondly, the authors have not studied the microbiological characteristics of the identified cases of septic spondylodiscitis after the lumbar spine PLDD procedure. And thirdly, several MRI images in the sagittal plane with artefacts, which may have affected the results of subcutaneous adipose tissue thickness calculations and, consequently, SAI, were included in the analysis.

CONCLUSION

The study showed that a high BMI value (more than 25 kg/m^2), as well as a high SAI value (over 0.7), were statistically significantly associated with the development of spondylodiscitis in patients had undergone a PLDD procedure for degenerative lumbar intervertebral cartilage disease. We believe that patients who have the above risk factors for dyscitis should be treated preoperatively with antibiotic prophylaxis, with careful treatment of the skin with antiseptic solutions, and that the puncture of the intervertebral cartilage should be performed with a slight retraction of the skin to create a "broken" surgical corridor. There is no doubt that large multicentre prospective studies including more respondents will be necessary to evaluate more objectively the proposed preventive measures for septic spondylodiscitis in patients who have undergone a lumbar PLDD.

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Conflict of interest

The authors declare the absence of a conflict of interest.

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ONCOLOGY

PRECURSORS, PATHWAYS OF CARCINOGENESIS AND MOLECULAR MARKERS OF VULVAR SQUAMOUS CELL CARCINOMA. LITERATURE REVIEW

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ABSTRACT

The review analyzes and summarizes the results of the studies on the pathogenesis of vulvar squamous cell carcinoma and its diagnostic features, reviews precursors and molecular subtypes of carcinomas. Despite the relatively low incidence of this tumor, over the past few decades, there has been an upward trend in its incidence, including the incidence among young women. According to the latest World Health Organization classification of lower genital tumors from 2020, vulvar squamous cell carcinoma is divided into human papillomavirus (HPV) associated and HPV-independent. While these carcinomas are often morphologically similar, their mechanisms of carcinogenesis, precursors, and clinical outcomes are different. Just the detection of virus DNA in a tumor is not enough to establish HPV status. Meanwhile immunohistochemical detection of the expression of p16 and p53 proteins allows not only to separate two pathogenetic pathways of carcinogenesis, but also to identify its molecular subtypes. The data on the possible use of p16 and p53 expression as the disease prognosis molecular markers have been obtained. Currently, the tactics of treatment and monitoring patients does not depend on the HPV status of carcinoma; however, the results of recent studies suggest that women with HPV positive vulvar cancer have significantly higher survival rates and a lower risk of recurrence. Understanding the mechanisms of carcinogenesis and improving its diagnosis will advance the assessment of the individual risk of the progression of precancerous lesions, as well as the outcome and the occurrence of tumor recurrence.

Key words: *vulvar squamous cell carcinoma, intraepithelial neoplasia, human papillomavirus, p16, p53*

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ПРЕДШЕСТВЕННИКИ, ПУТИ КАНЦЕРОГЕНЕЗА И МОЛЕКУЛЯРНЫЕ МАРКЕРЫ ПЛОСКОКЛЕТОЧНОЙ КАРЦИНОМЫ ВУЛЬВЫ. ЛИТЕРАТУРНЫЙ ОБЗОР

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РЕЗЮМЕ

В обзоре проанализированы и обобщены результаты исследований, изучающих патогенез плоскоклеточного рака вульвы и особенности его диагностики, рассмотрены предшествующие состояния и молекулярные подтипы карцином. Несмотря на относительно низкую встречаемость опухоли, за последние несколько десятилетий отмечается тенденция к росту заболеваемости, в том числе среди молодых женщин. Согласно последней классификации Всемирной организации здравоохранения опухолей нижнего отдела генитального тракта от 2020 г., плоскоклеточный рак вульвы подразделяется на ассоциированный с вирусом папилломы человека (ВПЧ) и ВПЧ-независимый. В то время как морфологически эти карциномы часто являются сходными, механизмы канцерогенеза, предшественники, а также клинические исходы у них различны. Только лишь обнаружение ДНК вируса в опухоли недостаточно для установки ВПЧ-статуса. В то же время иммуногистохимическое обнаружение экспрессии белков p16 и p53 позволяет не только разделить два патогенетических пути канцерогенеза, но и выявить его молекулярные подтипы. Получены данные о том, что экспрессию p16 и p53 возможно также использовать в качестве молекулярных маркеров прогноза заболевания. В настоящее время тактика лечения и наблюдения пациенток не зависит от ВПЧ-статуса карциномы, однако результаты исследований последних лет говорят о том, что женщины с ВПЧ-положительным раком вульвы имеют значительно более высокие показатели выживаемости и меньший риск возникновения рецидивов. Понимание механизмов канцерогенеза и усовершенствование его диагностики позволит улучшить оценку индивидуального риска прогресса предопухолевых состояний, а также исход и возникновение рецидива опухоли.

Ключевые слова: *плоскоклеточная карцинома вульвы, интраэпителиальная неоплазия, вирус папилломы человека, p16, p53*

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INTRODUCTION

Despite the fact that vulvar squamous cell carcinoma (VSCC) is a rare malignant tumor. In the last decade, the attention of an increasing number of scientists has been focused on studying its pathogenetic mechanisms, molecular events of carcinogenesis, as well as searching for reliable prognostic biomarkers of the disease [1–8]. In 2020, The World Health Organization has published a new classification of lower genital tumors, according to which vulvar squamous cell carcinoma (VSCC) are divided into human papillomavirus (HPV) associated and HPV-independent [9]. Unlike cervical squamous cell carcinoma, which in most cases is associated with HPV of high carcinogenic risk (HCR) [10–12], vulvar squamous cell carcinoma is more often HPV-independent; its molecular mechanisms are still poorly studied [13, 14]. Currently, the tactics of treatment and monitoring patients with an established diagnosis of VSCC depends only on the stage of the disease and somatic status [15, 16]. However, the results of large studies in recent years suggest that women with HPV-positive vulvar squamous cell carcinoma have significantly higher overall survival rates and a lower risk of recurrence [2, 6]. At the same time, a number of authors in their works do not come to the conclusion about the differences in prognosis depending on the mechanism of pathogenesis [17–19]. Differences in the results of studies may be due to the lack of a standardized set of methods for diagnosing HPV status. Despite the high sensitivity of the polymerase chain reaction (PCR) method, its results cannot be interpreted as sufficient, since only the detection of HPV DNA HCR is not a proof of its role in the process of malignancy [20, 21]. According to recent studies, immunohistochemical (IHC) detection of the expression of p16 and p53 proteins allows not only to separate the two pathogenetic pathways of carcinogenesis of the external genital organs [22–24], but also to use them as prognostic markers [5, 7, 25]. Thus, understanding the pathogenetic mechanisms, improving diagnostic methods, as well as determining the disease prognosis justify the need for a detailed and further study of the issue.

THE AIM OF THE REVIEW

To characterize the similarities and differences between HPV-associated and HPV-independent vulvar squamous cell carcinoma; to consider the probable and currently known carcinogenesis pathways, precursors, as well as the features of their diagnosis.

MOLECULAR MECHANISMS OF PATHOGENESIS OF TWO TYPES OF CARCINOMAS

HPV-associated carcinomas. Human papillomavirus is the most common sexually transmitted infection [12, 26]. However, in most cases it is transient, and subsequently the virus is eliminated from the body without transforming the cells [27]. Viral integration into the cell genome is an important event of carcinogenesis, leading to increased expression of the main viral E6 and E7 proteins, which in turn inactivate two tumor suppressors, namely p53 and retinoblastoma protein (pRb) [14, 27, 28]. E6 forms a triple complex with E6-associated protein (E6AP) and p53, altering the functional ability of p53 and causing degradation of this cellular protein through the ubiquitin-mediated proteolysis pathway, which leads to the absence of cell cycle arrest [29, 30]. E7 binds to pRb, which leads to the labeling of pRb for degradation, thereby releasing the E2F transcription factor from the pRb-E2F complex, which regulates cell proliferation, resulting in uncontrolled cell division [29, 31]. The Rb/E2F complex inhibits the transcription of several genes, including the *CDKN2A* gene encoding p16INK4a (p16). Consequently, the cleavage of Rb family members by the E7 protein leads to overexpression of p16 due to the release of the *CDKN2A* gene [14, 27]. An increase in the expression of the p16 protein occurs as an attempt to control uncontrolled cell division, which is mediated by a violation of the pRb pathway. However, the transduction of signals for the cell cycle arrest is also counteracted by E7-mediated activation of A and E cyclins [32]. Accelerated cell proliferation caused by high-risk HPV oncoproteins contributes to the accumulation of genetic defects, such as deletions, amplifications, translocations and chromosomal rearrangements, and leads to cell mutation [29].

HPV-independent carcinomas. In contrast to the relatively large amount of data on the pathogenesis of HPV-associated tumors, the HPV-independent pathway of VSCC has been studied much poorly, and the molecular mechanisms involved in the development of such VSCC have not yet been fully elucidated [14, 28]. According to studies, carcinomas etiologically unrelated to HPV more often contain mutations in the TP53 tumor suppressor gene. The product of this gene is the p53 protein (p53wt), whose function is to prevent DNA replication and trigger apoptosis in cells with abnormal DNA. This preserves the stability of the genome and prevents mutational changes [1, 7, 33]. Missense mutations in *TP53* lead to the accumulation of mutant p53 protein (p53mut) in cell nuclei, resistant to degradation and unable to perform its functions [7, 34]. According to studies, from 67 to 80 % of all HPV-independent tumors had mutations in this gene [1, 25, 35, 36]. At the same time, they are considered an early event of a carcinogen, since they were also detected in previous lesions [28, 37]. It is worth noting that mutations in the *TP53* gene can also occur in HPV-associated carcinomas, although much less frequently (about 25 % of cases). However, it is more likely that these changes are associated with the tumor progression, and not with the initiation of the process [29, 33].

Studies conducted using new generation sequencing methods have shown that *CDKN2A* is the second most frequently mutated gene, accounting for about 30–40 % of all genetic changes. As a result of its mutation, the p16 encoded by it is inactivated, which leads to disruption

of the cell cycle [18, 29, 33]. It is assumed that the combination of mutations in the *TP53* and *CDKN2A* genes correlates with a significantly worse prognosis of the disease [1, 38]. Also, *HRAS*, *PIK3CA*, *PTEN* and *NOTCH1* mutations were detected in HPV-independent carcinogenesis [8, 29, 39]. While some studies suggest that HPV-independent tumors have a large mutational load [25, 40], others [13, 39] indicated that the mutational load does not significantly depend on the HPV status. Perhaps this is due to differences in the methods of establishing the HPV status of the tumor and the use of different molecular markers.

In 2017, L.S. Nooij et al. in their work confirmed the third molecular subtype of VSCC independent of HPV and *TP53* mutation using genomic sequencing [25]. The group included 43 samples from a group of 236 subjects (18 %) and showed the highest frequency of *NOTCH1* and *HRAS* mutations. *NOTCH1* is a transmembrane receptor involved in cell differentiation and proliferation. The *HRAS* gene is involved in the PIK3CA/AKT/mTOR pathway, which regulates the processes of cell division and apoptosis. Their mutations can probably lead to the transduction of signals that activate cell proliferation and inhibit cell death. The mechanism of carcinogenesis in this group is difficult to understand and requires further study [22, 24]. Later, other authors also identified this subtype of tumors, but with different frequency of occurrence – from 4 % [41] and 10 % [22] to 25.7 % [23]. In the study by K.E. Kortekaas et al. 63 (15 %) cases from 413 studied samples were not associated with HPV and *TP53* mutation [5], while L. Woelber et al. obtained this subtype in 116 of 411 carcinomas (28 %) [24].

PREVIOUS CONDITIONS AND MORPHOLOGICAL FEATURES

Vulvar intraepithelial neoplasia. The development of invasive carcinoma is a slow, multi-stage process that many years [14]. Vulvar intraepithelial neoplasia (VIN) is a non-invasive squamous cell lesion and one of the most common precursors of vulvar squamous cell carcinoma. HPV-associated carcinomas usually develop from a high-grade squamous intraepithelial lesion (HSIL), previously known as usual vulvar intraepithelial neoplasia (uVIN), whereas HPV-independent arise from a precancerous lesion called differentiated VIN (dVIN) [42, 43].

According to studies, the detection rate of HSIL is significantly higher than dVIN (90–95 % vs. 5–10 %, respectively), but the probability of progression to invasive carcinoma is higher in the group of patients with diagnosed differentiated neoplasia. The risk of malignant transformation of the vulva is up to 15 % in women with unattended HSIL, below 5 % for HSIL after treatment and approximately 30 % for patients with dVIN [44, 45]. In addition, dVIN is associated with a shorter progression period. In the study of S.M. Bigby et al. the mean interval between dVIN biopsy and diagnosis of squamous cell carcinoma was 43.5 months (range 8–102 months) [46]. Similar indicators were shown in another study that included 1,826 women with HSIL and 67 women with dVIN; the latter had a higher risk of progression to malignant neoplasm (32.8 % vs. 5.7 %, respectively), which occurred on average after 22.8 months compared with 41.4 months for HSIL [47].

Morphological changes in HSIL are similar to all HPV-associated intraepithelial lesions, for example, as in high-grade cervical dysplasia. Dysplastic changes affect usually the entire thickness of the epithelium, are characterized by pronounced cellular polymorphism, as well as a violation of the nuclear-cytoplasmic ratio with nuclear hyperchromia. Koilocytic changes in the upper layers of the epithelium are characteristic [45]. Morphological diagnosis of dVIN presents certain difficulties due to indolent atypia, a high degree of differentiation of dysplastic cells, as well as due to the lack of accurate and reproducible diagnostic evaluation criteria. Para- and dyskeratosis, basal nuclear atypia of cells with an increase in size and nucleoli are characteristic. Koilocytic changes are not detected [44, 48]. However, there is evidence that some HPV-associated precursors mimic HPV-independent lesions, and, conversely, some HPV-independent intraepithelial precursors may have HSIL traits [25, 49].

Lichen sclerosus. Chronic inflammatory disease of autoimmune etiology, characterized morphologically by dermal lymphoid infiltration, collagen hyalinization and hyperkeratosis. Previously, lichen sclerosus (LS) was designated by the following terms: kraurosis, leukoplakia, sclerotic lichen or atrophic lichen [45]. It is assumed that the mechanism of occurrence of LS is the effect of activated T cells releasing interleukin-4 and transforming growth factor β on the cells of the basal layer. Thus, these cytokines activate fibroblasts, which leads to fibrosis. A long-term chronic process leads to the accumulation of genetic mutations [50, 51]. According to M.D. Trietsch et al., the frequency of *TP53* mutations in LS is 6 % [1]. In a study by L. Micheletti et al. among 976 women with LS, 34 (3.5 %) patients subsequently developed intraepithelial neoplasia, and 26 (2.7 %) had invasive squamous cell carcinoma [52]. Retrospective cohort studies show that LS is detected in the adjacent tissue from 5 to 88 % of invasive carcinomas, more often in combination with dVIN [4, 46, 53]. According to a recently published systematic review, the risk of developing VSCC in women with LS ranges from 3 to 21.8 %. Such variation is probably due to the wide variation in the occurrence of LS in studies, as well as diagnostic difficulties in differentiating LS from dVIN [54].

Vulvar aberrant maturation. A new general term for HPV-independent lesions combining vulvar aberrant maturation (VAM) with minimal nuclear atypia. It includes differentiated exophytic vulvar intraepithelial lesion (DEVIL), vulvar acanthosis with altered differentiation (VAAD), warty LS [50, 55, 56]. In 2017, J. Watkins et al. noted an association between verrucous (warty) and keratinizing squamous cell carcinoma, HPV-negative and many atypical warty lesions, many of which previously defied classification and did not meet the traditional criteria for vulvar intraepithelial neoplasia or other known prior lesions. They proposed the name DEVIL and identified these lesions based on the following signs: exophytic, with pronounced acanthosis or warty hyperplasia; ab-

sence of histomorphological characteristics of HSIL; absence of basal atypia sufficient to diagnose dVIN [36]. VAAD was described by A.F. Nascimento et al. back in 2004 [57], but taking into account similar histological features, J. Watkins et al. proposed to consider it as a form of DEVIL. They showed that these lesions demonstrate a lack of expression of abnormal p53, as well as a significant increase in the PIK3CA mutation. These data were confirmed later, in 2020, in the study of B. Tessier-Cloutier et al. [38]. Due to the rare occurrence, the malignancy potential of noninvasive warty vulvar lesions independent of HPV and *TP53* mutation is still unclear. However, it is likely that they are the precursors of the third molecular subtype of VSCC [55].

DIAGNOSTIC METHODS

Studies on the correlation between the morphological type of carcinoma and HPV status have shown that HPV-positive tumors were more often of the basaloid or warty type, whereas HPV-independent tumors were usually of the keratinizing type [20, 44]. According to a meta-analysis conducted by M.T. Faber et al., the prevalence of HPV DNA in the three main subtypes of VSCC is: 76.5 % – in warty carcinomas; 84.0 % – in basaloid carcinomas; 13.2 % – in the keratinizing type [58]. However, a large study by N. Rakislova et al., which included a large number of tissue samples (1594 paraffin blocks), showed that 36.5 % of HPV-associated carcinomas were of the usual keratinizing type [59]. In their work, A.S. Cheng et al. tried to classify VSCC as HPV-associated and independent of it, assessing the morphology of the tumor, the epithelial precursors adjacent to it and the age of the patients. Even using this multifaceted approach, they misclassified 17 % of cases regarding HPV status [20]. F. Dong et al. obtained similar data in their study, in which the results of molecular studies differed from the morphological assessment of HPV status in 21 % of cases [60]. These data once again emphasize the impossibility of reliably distinguishing HPV-associated and HPV-independent carcinomas only on the basis of morphological examination.

According to studies, immunohistochemical detection of p16 expression has demonstrated a high correlation with the detection of HPV DNA by PCR in cervical, anal, head and neck cancers and is considered a reliable marker of HPV-induced carcinogenesis [21, 61]. However, studies on the correlation between p16-positivity and HPV status in vulvar squamous cell cancer are controversial. Thus, in a large cohort multicenter study involving 1,709 samples, when testing for HPV DNA, the proportion of positive samples was 25.1 % ($n = 429$), while p16-positivity was observed only in 22.4 % ($n = 377$) [62]. J.J. Sznurkowsky et al. in their study showed that among 35 tissue samples with overexpression of p16, HPV DNA was absent in 10 cases (28.6 %), while among 50 tumors without overexpression of p16, HPV DNA was detected in 12 (24.0 %) cases [63]. The absence of DNA virus may be false negative, however, a highly sensitive PCR method with a wide range of primers (more than 68 types) was used in this study. The results obtained can probably be explained by the fact that the inactivation of Rb protein by E7, leading to the expression of p16, is only one potential form of the possible. Data were obtained showing a direct increase in p16 expression with chronological age. During the average life span, this protein increases approximately by 16 times [64, 65]. Thus, since VSCC is diagnosed more often in the age group of 65–70 years [3, 66], the expression of p16 may be due to the ageing rather than to the virus. The authors suggest not to consider only p16-positivity as a surrogate marker of HPV-positive status of VSCC [63].

The study by L. Woelber et al., representing part of a large retrospective study in Germany, showed that out of 411 tumor tissue samples, 204 (49.6 %) were HPV-positive DNA, while p16 expression was detected in 166 (30.2 %) samples. PCR was positive in 85.6 % of p16+ tumors (113 out of 132) and 32.3 % of all p16– tumors. Due to the unclear mutagenesis, cases of p16 discrepancy and HPV DNA detection were excluded from the analysis [24].

However, most authors have demonstrated a convincing correlation between a positive result for HPV DNA in samples and p16 expression with the method with 100 % sensitivity and 98 % specificity [20, 28, 67, 68]. In the study of G. Allo et al., 91 % of PCR-positive cases were also p16-positive; thus, sensitivity and specificity were 91 % and 84 %, respectively [69].

In some studies, the authors used the result of p16 expression as the only marker of the HPV status of VSCC [16, 41, 53]. Due to the fact that overexpression of p16 can occur individually and in different biological contexts, it may be necessary to assess the presence of both markers when determining the functional significance of HPV in carcinogenesis [21, 70].

Since *TP53* is the most frequently mutated gene in HPV-independent carcinogenesis, in 2020 B. Tessier-Cloutier et al. and K.E. Kortekaas et al. published studies showing a high correlation between the interpretation of p53 expression by the IHC method and its mutational status [71, 72]. According to their results, the IHC assessment of p53 expression in vulvar carcinoma samples differs from the interpretation of that in ovarian and endometrial carcinomas [73, 74]. Prior to their study, most authors in their works determined abnormal staining of p53, taking into account only the percentage of stained cells, and did not take into account their distribution among the epithelial layers [7, 60, 75–77]. The new study was able to demonstrate six different staining patterns: four displayed p53mut expression, and two displayed normal (wild) p53wt expression. The authors note that in cases of difficulty in assessing patterns, especially wild-type, interpretation of p53 staining in combination with p16 is necessary.

Thus, using the IHC analysis, three molecular subtypes of VSCC can be distinguished: 1) p16+/p53wt is associated with HPV infection; 2) p16-/p53mut, less frequently p16+/p53mut, corresponds to HPV-independent carcinoma with *TP53* mutation; 3) p16–/p53wt is HPV-independent and it is not associated with the *TP53* mutation. It should be noted that the IHC analysis is also useful for the differen-

tial diagnosis of VIN, since HSIL usually demonstrates block-positive staining of p16 and the expression pattern of p53wt, while dVIN does not show expression for p16 and demonstrates the pattern of mutant p53 [49, 50].

Oncoproteins p16 and p53 are the most studied biomarkers for VSCC diagnosis. The question of the expediency of determining the expression of PDL-1 in immune and tumor cells in vulvar cancer remains less studied. Studies have shown that the expression of this marker does not depend on the HPV status of the tumor. In addition, there is a discrepancy in the definition of expression in the primary and metastatic lesion of carcinoma. [23, 78]. The prognostic value of PD-L1 has not been sufficiently studied. However, the data obtained indicate that its positive expression is largely associated with metastasis of the process to the lymph nodes, as well as with the worst prognosis of overall survival among patients [23, 79].

The prognostic value of p16 and p53. Recent studies show that IHC-determination of p16 and p53 expression can also be used as molecular markers of disease prognosis. In his paper K.E. Kortekaas et al. have shown that the survival rates among the three molecular subtypes of squamous cell carcinomas are different [5]. The overall 5-year survival rate was 83 % (69,9–90,3 %), 64 % (48,9–75,9 %) and 48 % (41.5–55.0 %) for p16+/p53wt, p16–/p53wt and p16–/p53mut VSCC, respectively. A similar pattern was observed for relative survival. Among 275 p16–/p53mut cases there were 119 (43 %) recurrence compared to 16 of 63 (25 %) and 11 of 75 (15 %) for p16–/p53wt and p16+/p53wt, respectively ($p < 0.0001$). However, the time before the occurrence of recurrence did not differ between subtypes. L.S. Nooji et al. also demonstrated better overall and recurrence-free survival for patients with HPV-associated cancer, but the difference was not statistically significant [25]. The five-year survival rate was 75 %, 62.7 % and 56.3 % for patients with tumors p16+/p53wt, p16–/p53wt and p16-/p53mut, respectively ($p = 0.296$). The probability of recurrence was higher in the p16–/p53mut (22.6 %) and p16–/p53wt (16.3 %) groups compared to p16+/p53wt (5.3 %). In a recently published study, L. Woelber and colleagues also studied the clinical significance of p16 and p53 expression [24]. The two-year overall survival rates were 70.4 % (p16–/p53mut), 75.4 % (p16–/p53wt) and 82.5 % (p16+/p53wt) ($p = 0.005$). For two-year recurrence-free survival, the following indicators were obtained: 47.1 % (p16-/p53mut), 60.2 % (p16-/p53wt) and 63.9 % (p16+/p53wt) ($p < 0.001$). The risk of recurrence was 35.0 %, 32.0 % and 22.7 % for the p16–/p53mut, p16–/p53wt and 16+/p53wt groups, respectively. However, given the small number of articles evaluating the outcomes of all three molecular subtypes, further research in this direction is required.

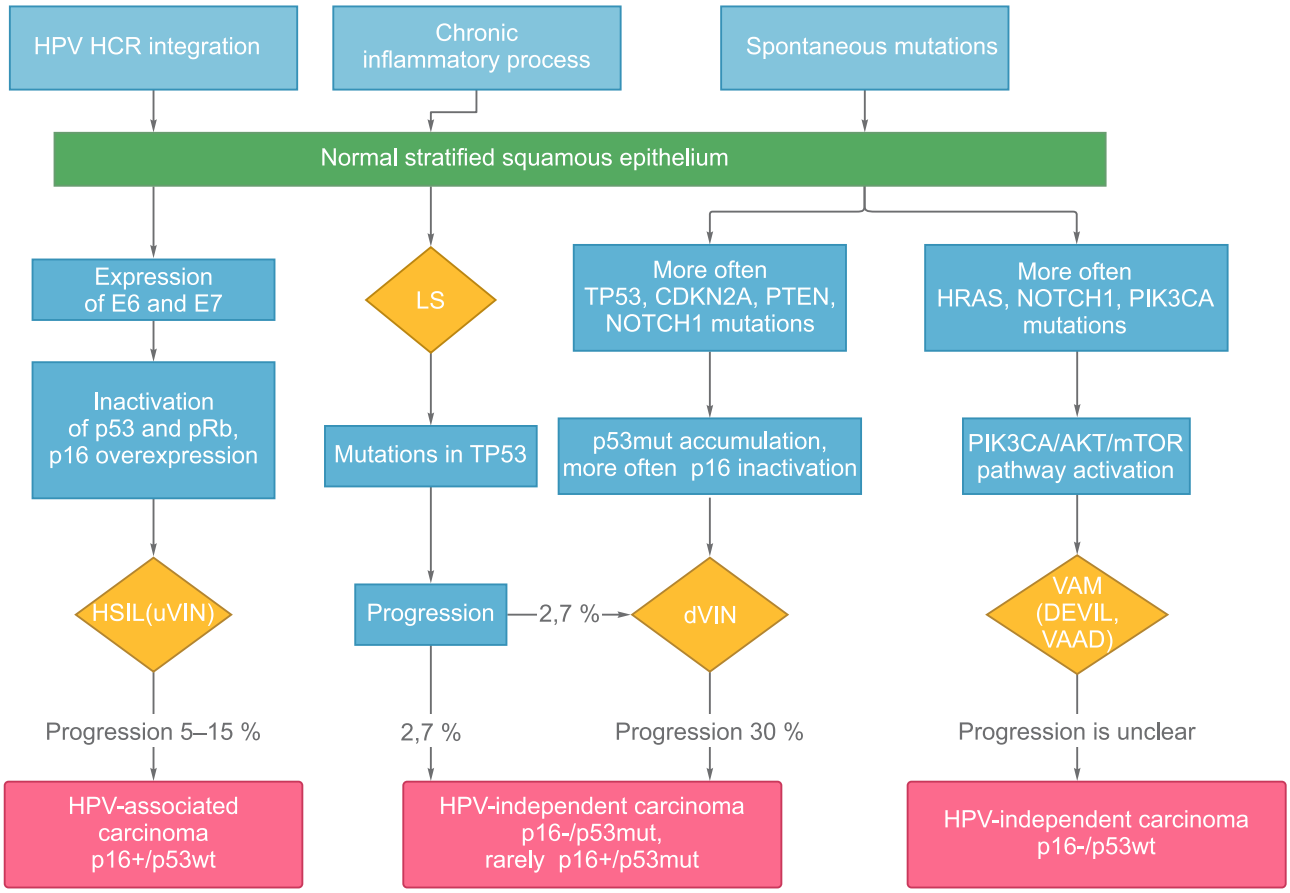

FIG. 1.

Precancerous lesions (HSIL, dVIN and VAM) and molecular subtypes of vulvar squamous cell carcinoma (HPV-associated and HPV-independent) diagnosed by immunohistological analysis of p16 and p53 expression

CONCLUSION

Vulvar squamous cell carcinoma can arise from previous lesions in several pathogenetic pathways, being HPV-associated or HPV-independent. Summarizing the literature data, we have drawn up a diagram of probable pathways of pathogenesis of VSCC (Fig. 1). While morphologically these carcinomas are often similar, the mechanisms of carcinogenesis and precursors, as well as clinical outcomes, are different. Immunohistochemical analysis is a common and affordable method that allows to determine the molecular subtype of carcinoma, as well as to improve the diagnosis of precancerous conditions of VSCC. At the same time, the use of p16 and p53 biomarkers as additional predictors of the outcome of the disease is of great interest. The question of finding new diagnostic and prognostic biomarkers of the disease, for example, PD-L1, remains open. The study of its role in the carcinogenesis of external genitalia deserves attention, since it provides the possibility of using new therapeutic approaches, such as inhibition of immune checkpoints. The possibility of targeting the PI3K/Akt/mTOR pathway with inhibitors should also be considered, whose mutations are common in both HPV-associated and HPV-independent carcinomas. However, given the rare incidence of this disease, further prospective multicenter studies are needed.

Conflict of interest

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

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OPHTALMOLOGY

FEATURES OF THE LOCAL CYTOKINE PROFILE OF PATIENTS WITH BULLOUS KERATOPATHY BY USING PERSONALIZED THERAPY WITH CELLULAR TECHNOLOGIES

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ABSTRACT

Background. *Today we have active development of ophthalmic surgery, but the role of cytokines in the pathogenesis of bullous keratopathy remains insufficiently studied at present.*

The aim. *To study the dynamics of the local cytokine profile in bullous keratopathy by using personalized treatment with suspension of autologous blood mononuclears.*

Material and methods. *Two groups of patients with bullous keratopathy were formed: the main group (30 people, 30 eyes), who received an intrastromal injection of a suspension of autologous blood mononuclears and comparison (28 people, 28 eyes), who received a course of pharmacotherapy. The level of interleukins (IL) – IL-10, IL-4, IL-6, IL-1ß, transforming growth factor beta-2 (TGF-β2), tumor necrosis factor alpha (TNF-α) was determined in the lacrimal fluid of the patient's eye by enzyme immunoassay.*

Results. *All patients before treatment showed an increase of IL-1ß in the lacrimal fluid by 3.3 times, IL-6 – by 4.2 times, TNF-α – by 2.0 times ($p < 0.05$); an increase in the level of IL-4, IL-10 and TGF-β2 by 1.1 times ($p > 0.05$). There was a decrease in IL-1ß by 2.0 times, IL-6 – by 2.1 times and TNF-α – by 1.8 times, and an increase in IL-10 by 1.5 times, IL-4 – by 1.9 times, TGF-β2 – by 1.4 times ($p < 0.05$) in the main group after treatment, persisting for 12 months. There was a short-term decrease of IL-1ß by 1.7 times, IL-6 and TNF-α – by 1.2 times and an increase of IL-10, IL-4, TGF-β2 by 1.2 times ($p < 0.05$) in the comparison group which then reached the initial values.*

Conclusion. *There is an imbalance in the system of pro- and anti-inflammatory cytokines in bullous keratopathy in the lacrimal fluid. Injection of autologous mononuclear cells in the stroma of the cornea reduces the severity of the imbalance of the local cytokine system compared to the course of pharmacotherapy.*

Key words: *bullous keratopathy, cellular technologies, autologous mononuclear leukocytes, cytokines*

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ДИНАМИКА ЛОКАЛЬНОГО ЦИТОКИНОВОГО ПРОФИЛЯ ПРИ ЭНДОТЕЛИАЛЬНО-ЭПИТЕЛИАЛЬНОЙ ДИСТРОФИИ РОГОВИЦЫ НА ФОНЕ ПЕРСОНИФИЦИРОВАННОЙ ТЕРАПИИ С ИСПОЛЬЗОВАНИЕМ КЛЕТОЧНЫХ ТЕХНОЛОГИЙ

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РЕЗЮМЕ

Обоснование. *Активное развитие офтальмохирургии обусловливает увеличение числа пациентов с эндотелиально-эпителиальной дистрофией (ЭЭД) роговицы, роль цитокинов в патогенезе которой остаётся недостаточно изученной.*

Цель исследования: *изучить динамику локального цитокинового профиля при ЭЭД роговицы в условиях персонифицированного лечения с использованием суспензии аутологичных мононуклеаров крови.*

Материал и методы. *Сформировано две группы больных с ЭЭД роговицы: основная (30 человек, 30 глаз), получившая интрастромальное введение суспензии аутологичных мононуклеаров крови, и сравнения (28 человек, 28 глаз), получившая курс фармакотерапии. В слёзной жидкости (СЖ) больного глаза определяли уровень интерлейкинов (IL) – IL-10, IL-4, IL-6, IL-1β, трансформирующего фактора роста бета-2 (TGF-β2, transforming growth factor β2), фактора некроза опухоли альфа (TNF-α, tumor necrosis factor α) методом иммуноферментного анализа.*

Результаты. *У всех пациентов до лечения в СЖ выявлено повышение IL-1β в 3,3 раза, IL-6 – в 4,2 раза, TNF-α – в 2,0 раза ($p < 0,05$); увеличение уровней IL-4, IL-10 и TGF-β2 в 1,1 раза ($p > 0,05$). В основной группе после лечения отмечено снижение IL-1β в 2,0 раза, IL-6 – в 2,1 раза и TNF-α – в 1,8 раза; повышение IL-10 в 1,5 раза, IL-4 – в 1,9 раза, TGF-β2 – в 1,4 раза ($p < 0,05$), сохраняющееся в течение 12 мес. В группе сравнения отмечено краткосрочное снижение IL-1β в 1,7 раза, IL-6 и TNF-α – в 1,2 раза; увеличение уровней IL-10, IL-4, TGF-β2 в 1,2 раза ($p < 0,05$), которые затем достигли первоначальных значений.*

Заключение. *Выявлен дисбаланс в СЖ в системе про- и противовоспалительных цитокинов при ЭЭД роговицы. Интрастромальное введение аутологичных мононуклеров уменьшает выраженность дисбаланса локальной цитокиновой системы по сравнению с курсом фармакотерапии.*

Ключевые слова: *эндотелиально-эпителиальная дистрофия роговицы, клеточные технологии, аутологичные мононуклеарные лейкоциты, цитокины*

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RELEVANCE

Bullous keratopathy or endothelial-epithelial dystrophy of the cornea is a progressive disease of the cornea, the main manifestation of which is a chronic corneal edema and a pain syndrome. According to modern concepts [1–3], the pathogenesis is based on a decrease in the density of endothelial cells of the cornea, the cause of which may be injury, previous surgery or ocular inflammatory diseases. These changes lead to an increase in the permeability of the endothelial layer of the cornea, followed by hydration of the corneal stroma and keratocyte dystrophy [4, 5]. More and more data on the role of cytokines in the pathogenesis of bullous keratopathy have appeared in recent scientific publications [6-8]. However, these facts are currently fragmented, which makes it necessary to study in detail the local cytokine status in bullous keratopathy and develop new methods of treating this pathology of the cornea using cellular technologies.

THE AIM

To study the dynamics of the local cytokine profile in bullous keratopathy by using personalized treatment with suspension of autologous blood mononuclears.

MATERIAL AND METHODS

The presented clinical study was carried out at the Ophthalmology Clinic and the Central Research Laboratory of Siberian State Medical University (Tomsk). 58 patients with an established diagnosis of bullous keratopathy were under observation. The study was conducted in two stages. At the stage I of the study, the cytokine profile of the lacrimal fluid was studied in patients with bullous keratopathy. At the stage II the developed method of surgical treatment of bullous keratopathy using cellular technologies was tested in clinical practice and its effectiveness was assessed in comparison with pharmacotherapy. The approval of the clinical study was carried out by the local ethics committee of the Siberian State Medical University (registration number No. 5598, dated 06.11.2017). All patients signed a voluntary informed consent before the treatment.

The inclusion criteria: established diagnosis of bullous stage of bullous keratopathy (according to the classification of V.V. Volkov, M.M. Dronov, 1978), age from 18 to 85 years old. The exclusion criteria: general somatic diseases in the decompensation stage, mental illnesses, age under 18 years old, acute and chronic ocular inflammatory diseases in the exacerbation stage, monophthalm, pregnancy and lactation.

To study the local cytokine profile in bullous keratopathy in patients (58 patients, 58 eyes), the lacrimal fluid was taken from the inferior conjunctival fornix of the sore eye at the stage I of the clinical study. An automatic pipette was used to collect biological material. The lacrimal fluid was taken within 10 minutes from an inferior conjunctival fornix without prior stimulation. To study the samples obtained by enzyme immunoassay (ELISA), the following types of cytokines were selected: interleukins (IL) – IL-4, IL-10, IL-1β, IL-6, tumor necrosis factor α (TNF-α), transforming growth factor β2 (TGF-β2). Cytokine content was determined according to the attached instructions to the test systems: IL-10, IL-1β, IL-4, IL-6, TNF-α – Vector Best (Russia), TGF-β2 – Bender MedSystems (Austria). The ELISA results were recorded on a Uniplan vertical photometer (Russia) at a wavelength of 450 nm.

For the stage II of the clinical study, all patients (58 people), depending on the planned treatment, were divided into two groups: the main group and the comparison group. The main group of patients (30 people, 30 eyes) received personalized surgical treatment of bullous keratopathy by creating a local detachment of the Descemet's membrane due to the injection of sterile air into the posterior third of the cornea, followed by the injection of 0.3–0.4 ml of freshly isolated suspension of autologous mononuclear blood leukocytes into the area of the detached Descemet's membrane (patent No. 2674237 dated 05.12.2018). The surgical manipulation was performed once. In the postoperative period, patients received instillations of 0.3% solution of tobramycin 4 times a day for 7 days in order to prevent secondary infection.

Patients of the comparison group (28 people, 28 eyes) received a course of pharmacotherapy, including subconjunctival injections of 0.01% solution of riboflavin 1 time a day, instillations of 0.01% aqueous solution of vitamin B2, 5% dexapanthenol gel 4 times a day for 14 days.

The lacrimal fluid sampling from the conjunctival cavity of the sore eye in patients of both groups was carried out in the described way on the 3rd, 10th day from the onset of the treatment, as well as 1, 3, 6, 9 and 12 months after the treatment. The obtained lacrimal fluid samples were frozen at –20 °C, which made it possible to use them for three months.

The total duration of the study was 12 months.

Statistical processing of the results was carried out using the Statistica 13.3 program (StatSoft Inc., USA). After testing for normality of distribution using the Kolmogorov-Smirnov test the differences of dependent variables within the group were assessed using Wilcoxon's t-test, and the differences between the groups were assessed using Mann – Whitney U test. The results of this work are presented in the form $M \pm m$, where M is the sample mean, m is the error of the mean. Differences in indicators were considered statistically significant at the level of $p < 0.05$.

RESULTS

The gender and age distribution of patients was as follows. The main group included 30 patients, including 28 women and 2 men with an average age of 79.4 years, and in the comparison group – 28 people, including 27 women and 1 man ($p > 0.05$) with an average age of 77.7 years ($p > 0.05$).

According to the results of the study of the local cytokine profile in patients with bullous keratopathy, an increase in the content of proinflammatory cytokines (IL-1ß, IL-6, TNF-α) was detected in the samples of the the lacrimal fluid before the treatment. Thus, the baseline of IL-1ß in both

groups was increased by 3.3 times compared to the normal value ($p < 0.05$) [9], the level of IL-6 in both groups was increased by 4.2 times ($p < 0.05$) [10], the TNF-α index in both groups was 2.0 times higher than the normal value ($p < 0.05$) [11] (Tables 1, 2). There were no statistically significant differences in the indicators of these cytokines in patients of the main group and the comparison group before the treatment ($p > 0.05$) (Tables 1, 2).

The baseline level (before treatment) of IL-10, IL-4, TGF-β2 in the lacrimal fluid of the sore eye in all patients before the treatment was 1.1 times higher than the normal value [10, 12, 13], which, however, was not statistically significant ($p > 0.05$) (Tables 1, 2).

According to the results of the stage II of the study, on the 3rd day from the onset of the treatment, patients of both groups showed a slight, not statistically significant, decrease in the level of IL-1ß in the samples of the lacrimal fluid of the sore eye compared with the baseline ($p > 0.05$) (Tables 1, 2). On the 10th day after treatment with a new surgical method with using cellular technologies in patients of the main group, the level of this cytokine in the lacrimal fluid of the sore eye decreased by 1.7 times ($p < 0.05$) from the baseline (Table 1), while in patients of the comparison group who received conservative treatment – by 1.3 times ($p < 0.05$) (Table 2). 1 month after intrastromal injection of a suspension of autologous blood mononuclears the maximum decrease in the level of IL-1ß in the lacrimal fluid in patients of the main group during the entire observation period was 2.0 times compared with the baseline ($p < 0.05$) (Table 1), in patients of the comparison group – by 1.7 times from the baseline

TABLE 1

THE DYNAMICS OF CYTOKINE LEVEL IN LACRIMAL FLUID IN PATIENTS OF THE MAIN GROUP BEFORE AND AFTER THE TREATMENT, PG/ML (M ± m)

Follow-up period	Cytokines					
	IL-1β	IL-6	TNF-α	IL-4	IL-10	TGF-β2
Standard	18.2 ± 3.5	125.3 ± 11.7	252.6 ± 11.1	26.3 ± 7.4	142.4 ± 5.0	168.5 ± 25.3
Before the treatment	59.3 ± 9.5	523.6 ± 16.8	497.6 ± 20.5	29.8 ± 2.7	156.4 ± 3.3	184.4 ± 8.3
3rd day	56.4 ± 7.8*	420.3 ± 18.6*	354.8 ± 28.8*	69.4 ± 4.8*	275.4 ± 5.2*	207.6 ± 8.9
10th day	34.2 ± 5.2*	336.8 ± 24.6*	320.4 ± 23.7*	45.4 ± 3.8*	294.3 ± 5.6*	205.8 ± 11.2
1 m	29.8 ± 3.3*	305.4 ± 23.3*	294.8 ± 19.2*	42.4 ± 1.6*	286.9 ± 4.4*	258.1 ± 16.1*
3 m	33.4 ± 4.9*	254.3 ± 24.7*	284.3 ± 18.94*	39.5 ± 1.4*	296.3 ± 3.7*	245.4 ± 14.7*
6 m	32.5 ± 3.7*	246.8 ± 25.3*	275.3 ± 17.5*	40.4 ± 1.5*	244.8 ± 4.0*	179.5 ± 7.9
9 m	34.6 ± 3.8*	236.7 ± 23.8*	279.6 ± 15.9*	44.7 ± 1.4*	232.1 ± 2.2*	180.3 ± 8.4
12 m	34.9 ± 3.1*	243.8 ± 21.3*	278.4 ± 13.3*	43.2 ± 1.3*	230.3 ± 1.8*	182.4 ± 8.9

Note. * – the level of statistical significance of the differences compared to the baseline ($p < 0.05$).

TABLE 2

THE DYNAMICS OF CYTOKINE LEVEL IN LACRIMAL FLUID IN PATIENTS OF THE COMPARISON GROUP BEFORE AND AFTER THE TREATMENT PG/ML (M ± m)

Follow-up period	Cytokines					
	IL-1β	IL-6	TNF-α	IL-4	IL-10	TGF-β2
Standard	18.2 ± 3.5	125.3 ± 11.7	252.6 ± 11.1	26.3 ± 7.4	142.4 ± 5.0	168.5 ± 25.3
Before the treatment	58.4 ± 7.9	519.6 ± 12.2	502.4 ± 10.5	30.2 ± ,3.0	158.4 ± 4.3	179.7 ± 6.1
3rd day	57.2 ± 7.1	495.7 ± 10.1*	482.3 ± 12.0	33.5 ± 3.4	162.5 ± 4.3	189.3 ± 9.0
10th day	45.3 ± 6.7*	478.5 ± 8.0	435.3 ± 17.7*	35.6 ± 3.7*	189.4 ± 6.8*	195.4 ± 9.6
1 m	35.6 ± 6.1*	450.6 ± 13.5*	472.8 ± 18.4	31.3 ± 3.3	162.4 ± 5.1	215.3 ± 8.5*
3 m	39.7 ± 4.9*	487.5 ± 17.6	485.3 ± 15.9	27.8 ± 2.8	154.3 ± 5.4	188.3 ± 7.6
6 m	49.3 ± 4.7*	501.7 ± 13.4	492.3 ± 10.2	26.9 ± 2.7	157.5 ± 6.5	175.2 ± 8.9
9 m	53.4 ± 4.5	510.7 ± 11.2	489.6 ± 7.4	27.5 ± 3.0	156.1 ± 5.6	172.8 ± 9.4
12 m	54.7 ± 5.9	517.7 ± 10.4	485.3 ± 7.3	26.4 ± 2.3	149.8 ± 5.9	184.3 ± 8.2

Note. * – the level of statistical significance of the differences compared to the baseline ($p < 0.05$).

($p < 0.05$) (Table 2). In 3 months the level of IL-1ß in the lacrimal fluid of the sore eye in the patients of the main group increased slightly to 33.4 pg/ml ($p > 0.05$), but subsequently remained stable throughout the follow–up (Table 1). The level of this cytokine in the lacrimal fluid of the sore eye in patients of the comparison group gradually increased during observation and reached the baseline before treatment 12 months after the course of pharmacotherapy (Table 2).

On the 3rd day after the presented surgical treatment the level of IL-6 in the lacrimal fluid of the sore eye in the main group of patients decreased by 1.3 times compared to the baseline ($p < 0.05$) (Table 1), while in patients of the comparison group this indicator decreased by 1.1 times ($p > 0.05$) (Table 2). 1 month after the treatment the level of this cytokine in the main group decreased by 1.7 times from the baseline ($p < 0.05$) (Table. 1), in the comparison group – only 1.2 times ($p < 0.05$) (Table 2). 3 months after intrastromal injection of a suspension of autologous blood mononuclears the content of IL-6 in the lacrimal fluid of the sore eye in patients of the main group decreased by 2.1 times ($p < 0.05$) from the baseline, and subsequently its stabilization was noted throughout the entire follow-up period (Table 1). The level of this cytokine in patients of the comparison group began to gradually increase 3 months after the course of pharmacotherapy and reached the baseline after 12 months (Table 2).

The level of TNF-α in the lacrimal fluid of the sore eye in patients of the main group on the 3rd day after the injection of a suspension of autologous blood mononuclears into the stroma of the cornea decreased by 1.4 times ($p < 0.05$) from the baseline (Table. 1), while in the comparison group against the background of conservative treatment – only 1.1 times ($p > 0.05$) (Table 2). The TNF-α level in patients of the main group decreased 1.6 times from the baseline on the 10th day after the onset of the treatment ($p < 0.05$) (Table 1), in the comparison group – by 1.2 times ($p < 0.05$) (Table 2). The level of TNF-α in the lacrimal fluid in the main group continued to decrease during further follow-up and reached the minimum value by the 6th month of follow-up (Table 1). In the comparison group, on the contrary, a gradual increase in the content of this cytokine was noted (Table 2).

The content of IL-4 in the lacrimal fluid in patients of the main group with bullous keratopathy on the 3rd day after the described surgical treatment increased 2.3 times ($p < 0.05$) from the baseline (Table 1), while in patients of the comparison group who received conservative treatment it was only 1.1 times ($p > 0.05$) (Table 2). On the 10th day after intrastromal injection of a suspension of autologous mononuclears in patients of the main group the level of the studied cytokine exceeded the baseline by 1.5 times ($p < 0.05$) (Table 1), in patients of the comparison group – by 1.2 times ($p < 0.05$) (Table 2). 1 month after treatment the content of IL-4 in the lacrimal fluid of the sore eye in patients of the main group exceeded the baseline by 1.4 times ($p < 0.05$). At the same time, this indicator remained relatively stable throughout the entire follow-up period (12 months) (Table 1). 1 month after the course of pharmacotherapy the content of IL-4 in the lacrimal fluid of the sore eye in patients of the comparison group did not significantly differ from that before the treatment, and subsequently, its gradual decrease was observed within 12 months (Table 2).

The level of IL-10 in the lacrimal fluid of the sore eye in patients of the main group on the 3rd day after the injection of a suspension of autologous mononuclears into the stroma of the cornea increased by 1.7 times from the baseline ($p < 0.05$) (Table 1). There was no statistically significant change in this cytokine in patients of the comparison group (Table 2). On the 10th day after surgical treatment with using cellular technologies the level of IL-10 in the lacrimal fluid in patients of the main group increased by 1.8 times from the baseline ($p < 0.05$) (Table 1), in the comparison group – by 1.2 times ($p < 0.05$) (Table 2). After 1 month after surgical treatment the level of this cytokine in the lacrimal fluid of the sore eye in patients of the main group exceeded the baseline by 1.7 times ($p < 0.05$), and after 3 months – by 1.9 times ($p < 0.05$). Stabilization of the level of IL-10 in the lacrimal fluid of the sore eye was revealed during further follow-up (up to 12 months) after a slight, non-statistically significant decrease ($p > 0.05$) compared with the indicator after 6 months (Table 1). 10 days after the onset of pharmacotherapy a statistically significant increase (1.2 times; $p < 0.05$) in the level of this cytokine in the lacrimal fluid of the sore eye in the comparison group was noted, and by the end of observation (12 months) its content in the lacrimal fluid corresponded to that before treatment (Table 2).

The level of TGF-β2 in the lacrimal fluid of the sore eye on the 3rd day after treatment in patients of both groups increased only 1.1 times from the baseline (Tables 1, 2), which is not statistically significant ($p > 0.05$). This tendency persisted in both groups (Tables 1, 2) on the 10th day after treatment. After 1 month the level of TGF-β2 in the lacrimal fluid of the sore eye in patients of the main group increased by 1.4 times ($p < 0.05$) from the baseline (Table 1), in the comparison group – by 1.2 times ($p < 0.05$) (Table 2). Further follow-up revealed a gradual decrease in the level of TGF-β2 in both groups, which reached the baseline by 12 months after the treatment (Tables 1, 2).

DISCUSSION

The analysis of the obtained results allows to detect a statistically significant increase in the lacrimal fluid of the sore eye of proinflammatory cytokines IL-1ß, IL-6, TNF-α in all patients with bullous keratopathy before the treatment, which indicates a significant role of inflammation in the pathogenesis of this disease. Thus, IL-1ß is a cytokine that induces inflammation at the local level and increases the chemotaxis of effector cells to the pathological focus [14]. IL-6 also contributes to the activation of inflammation against the background of a decrease in the control function of excessive inflammatory response [15], and TNF-α is one of the most significant proinflammatory cytokines and participates in the pathogenesis of many immuno-inflammatory diseases. TNF-α increases the functional activity of leukocytes and macrophages, enhancing phagocytosis and cytokine synthesis [15].

At the same time, a slight increase in the content of cytokines such as IL-10 and -4, TGF-β2, which is not statistically significant ($p > 0.05$), was detected in the lacrimal fluid of the sore eye in all patients with bullous keratopathy before the treatment. These biologically active substances are multifunctional cytokines that regulate a large number of biological processes, such as migration, proliferation and apoptosis of various types of cells, and have anti-inflammatory and immunosuppressive effects [14, 15, 16]. The features of the local cytokine profile revealed during the study in bullous keratopathy indicate the presence of an imbalance in the system of pro- and anti-inflammatory cytokines, the severity of which to a certain extent may determine the severity of the course of dystrophic lesions of the cornea.

According to the results of the stage II of the study, the injection of a suspension of autologous blood mononuclears into the stroma of the cornea during bullous keratopathy as a source of cytokines contributes to the gradual restoration (within three months) of the level of the studied cytokines in the lacrimal fluid of the sore eye, reducing the existing imbalance. This, in turn, provides relief of inflammation with stabilization of the pathological process in the dystrophically altered cornea.

The course of conservative treatment carried out in patients of the comparison group, according to the data obtained, provides only a short-term reduction (within 1 month) of the imbalance in the local cytokine system in bullous keratopathy with subsequent regression.

CONCLUSIONS

There is an imbalance in the system of pro- and anti-inflammatory cytokines in the pathogenesis of bullous keratopathy. And this imbalance to a certain extent determines the severity of the disease.

The new personalized surgical method of treatment of bullous keratopathy with using cellular technologies allows to reduce the severity of the imbalance of the local cytokine system in comparison with the course of pharmacotherapy, ensuring the stabilization of the pathological process.

Conflict of interest

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

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RESULTS OF SURGICAL TREATMENT OF PTOSIS OF THE UPPER EYELID BY THE DOSED MUSCLE-CONJUNCTIVAL RESECTION OF THE UPPER CARTILAGE

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ABSTRACT

Blepharoptosis is an abnormally low position of the upper eyelid in relation to the eyeball due to its omission. The method of surgical treatment of blepharoptosis depends on the function of the levator.

The aim. *To analyze the results of surgical treatment of partial blepharoptosis of various etiology by the dosed muscle-conjunctival resection of the upper eyelid cartilage.*

Material and methods. *We operated 78 patients (82 eyes) with blepharoptosis. Among them with congenital blepharoptosis – 34 people (36 eyes), with acquired – 44 (46 eyes). The patients' age was from 6 to 82 years.*

Inclusion criteria: the function of the upper eyelid levator – more than 5 mm, the width of the palpebral fissure – 3 mm or more.

The patients underwent a dosed muscle-conjunctival resection of the upper eyelid cartilage in the period from 2018 to 2021. The follow-up period ranged from 2 months to 2 years.

Results. *Elimination of blepharoptosis was achieved in all patients. With congenital blepharoptosis, a good result was obtained in 33 people (91.7 %), satisfactory – in three patients (8.3 %), which was associated with the asymmetry of the palpebral fissure, for the correction of which these patients underwent additional intervention. In patients with acquired ptosis of the upper eyelid, a good result was achieved in 91.3 % of cases (42 eyes). One patient underwent repeated surgery due to hypocorrection and asymmetry of the palpebral fissure width after the first surgery.*

In all patients, the result remained stable throughout the observation period.

Conclusion. *The use of the method of dosed muscle-conjunctival resection of the upper eyelid cartilage in patients with partial ptosis of the upper eyelid and intact levator function made it possible to obtain a good cosmetic and functional result: with congenital blepharoptosis – in 91.7 % of cases, acquired ptosis of the upper eyelid – in 91.3 % patients.*

Key words: *upper eyelid ptosis, cartilage resection, blepharoptosis, upper eyelid levator function*

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РЕЗУЛЬТАТЫ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ ПТОЗА ВЕРХНЕГО ВЕКА МЕТОДОМ ДОЗИРОВАННОЙ МЫШЕЧНО-КОНЪЮНКТИВАЛЬНОЙ РЕЗЕКЦИИ ХРЯЩА ВЕРХНЕГО ВЕКА

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РЕЗЮМЕ

Блефароптоз – аномально низкое положение верхнего века по отношению к глазному яблоку вследствие его опущения. Метод хирургического лечения блефароптоза зависит от функции леватора.

***Цель исследования**: анализ результатов хирургического лечения частичного блефароптоза различной этиологии методом дозированной мышечно-конъюнктивальной резекции хряща верхнего века.*

***Материал и методы**. Прооперировано 78 пациентов (82 глаза). Врождённый блефароптоз был у 34 человек (36 глаз), приобретённый – у 44 (46 глаз). Возраст пациентов – от 6 до 82 лет. Критерии включения: функция леватора верхнего века более 5 мм, ширина глазной щели 3 мм и более. Пациентам проведена дозированная мышечно-конъюнктивальная резекция хряща верхнего века в период с 2018 по 2021 г. Срок наблюдения – от 2 месяцев до 2 лет.*

***Результаты**. У всех пациентов достигнуто устранение блефароптоза. При врождённом блефароптозе хороший результат был получен в 33 случаях (91,7 %), удовлетворительный – у трёх пациентов (8,3 %), что было связано с асимметрией глазной щели, для коррекции которой данным больным проведено дополнительное вмешательство.*

У пациентов с приобретённым блефароптозом хороший результат достигнут в 91,3 % случаев (42 глаза). Одному больному проведено повторное хирургическое вмешательство вследствие гипокоррекции и асимметрии ширины глазной щели.

У всех пациентов результат оставался стабильным на протяжении всего срока наблюдения.

***Заключение.** Применение дозированной мышечно-конъюнктивальной резекции хряща верхнего века у пациентов с частичным птозом верхнего века и сохранной функцией леватора позволило получить хороший косметический и функциональный результат: при врождённом блефароптозе – в 91,7 % случаев, приобретённом – в 91,3 %.*

***Ключевые слова**: птоз верхнего века, резекция хряща, блефароптоз, функция леватора верхнего века*

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RELEVANCE

Ptosis of the upper eyelid (blepharoptosis) is an abnormal drooping of the upper eyelid due to insufficient function of the muscle that raises the upper eyelid (levator) [1]. Blepharoptosis is one of the most common congenital ophthalmopathologies. Drooping of the upper eyelid is not only a significant cosmetic defect, but can also be the cause of such serious conditions as obscuration amblyopia, strabismus, binocular vision disorder, postural disorder, abnormal head posture [2–4].

During the treatment of ptosis of the upper eyelid, conservative methods practically do not give any results. Surgical treatment of blepharoptosis is the most effective [5, 6]. The choice of the method of surgical treatment of ptosis of the upper eyelid depends on the function of the levator. Resection of the muscle that raises the upper eyelid is performed if the function of the muscle is reduced, but intact, a suspension-type surgery is performed in case of its absence.

Currently, the literature describes many methods of surgical treatment of ptosis of the upper eyelid, such as transcutaneous resection of the muscle lifting the upper eyelid, shortening of the levator by performing reduplication, suspension of the muscle lifting the upper eyelid to the frontal muscle using various materials for suspension [7, 8]. In particular, a group of authors proposed a method of surgical treatment of blepharoptosis consisting of dosed muscle-conjunctival resection of the upper eyelid cartilage [1, 9–11]. Despite the development of numerous methods and types of surgery aimed at correcting the upper eyelid drooping, none of the methods can completely solve the problem of this condition. In this regard, it seems appropriate to evaluate the results of surgical treatment of partial blepharoptosis of various etiology by this method.

THE AIM OF THE STUDY

To analyze the results of surgical treatment of partial blepharoptosis of various etiology by the dosed muscle-conjunctival resection of the upper eyelid cartilage.

MATERIAL AND METHODS

78 patients (82 eyes) with the upper eyelid ptosis of various etiology were operated: among them with congenital blepharoptosis – 34 people (36 eyes), with acquired – 44 people (46 eyes). 74 patients had unilateral upper eyelid ptosis, 4 patients – bilateral. 23 patients (23 eyes) had mild blepharoptosis, 55 people (59 eyes) – moderate [12]. According to the degree of the upper eyelid drooping, the groups of patients with congenital and acquired ptosis were comparable. The patients' age was from 6 to 82 years.

Inclusion criteria: the function of the upper eyelid levator – more than 5 mm, the width of the palpebral fissure – 3 mm or more. Exclusion criteria: the function of the upper eyelid levator – 5 mm or less, the width of the palpebral fissure – less than 3 mm, inflammatory eye conditions.

The patients underwent a standard preoperative ophthalmological examination, including visometry, biomicroscopy, ophthalmoscopy, tonometry, keratometry and refractometry. The width of the palpebral fissure, the height of the upper eyelid relative to the pupil, the function of the upper eyelid levator were also determined; MRD1 (marginal reflex distance 1) was evaluated, the presence or absence of the Bell's phenomenon (deviation of the eyeballs upward when closing the eyelids) was determined. The amplitude of the upper eyelid movement was measured in millimeters, when looking up or down as much as possible, with and without the frontal muscle fixation by a linear method in order to determine the function of the muscle that raises the upper eyelid. Surgical treatment of the patients was carried out in the period from 2018 to 2021. The follow-up period ranged from 2 months to 2 years.

Elimination of blepharoptosis was carried out by the method of the dosed muscle-conjunctival resection of the upper eyelid cartilage. Dosing was carried out depending on the function of the levator and the level of the upper eyelid margin in relation to the limbus.

The surgery technique. The upper eyelid is turned out, the level of resection of the cartilage of the upper eyelid cartilage is marked. The cartilage forceps is applied. The conjunctiva, cartilage of the upper eyelid, levator, Müller's muscle from the temporal part to the nasal part and in the opposite direction is stitched with Vicryl 6/0 U-shaped sutures. The ends of the suture are displayed on the skin of the temporal area. The distance from the sutures to the forceps shall not be more than 1 mm. The tissues of the upper eyelid (conjunctiva, upper eyelid cartilage, upper eyelid lifting muscle, Müller's muscle), fixed between the branches of the forceps, are excised according to the marking. A continuous Vicryl 6/0 suture is applied to the resected margin of the cartilage.

In the postoperative period, patients underwent antibacterial, anti-inflammatory treatment, ointment keratoprotectors were used to prevent the development of keratopathy.

The effectiveness of the surgical treatment in the postoperative period was assessed by the level of the upper eyelid margin in relation to the pupil. With unilateral ptosis, the result was considered good if the width of the palpebral fissure became symmetrical, and satisfactory – with a difference in the width of the palpebral fissure up to 1.0–1.5 mm. In bilateral blepharoptosis, the result was good when the position of the upper eyelid was at the level of the upper limbus or above the upper margin of the pupil, and the width of the palpebral fissure in both eyes was symmetrical. The result was considered satisfactory if the upper eyelid was located at the level of the upper margin of the pupil.

Statistical processing of the analyzed data was performed using the Statistica program, version 8.0 (Stat-

TABLE 1

ASSESSMENT OF THE FUNCTION OF THE LEVATOR OF THE UPPER EYELID BEFORE AND AT DIFFERENT TIMES AFTER SURGERY (MRD1)

	MRD1 (1 mm)				Upper eyelid excursion (mm)			
	Before treatment	After treatment			Before treatment	After treatment		
		1m	6m	12m		1m	6m	12m
Congenital (n = 36)	1.8 ± 0.7	4.9 ± 0.9*	4.8 ± 0.7*	4.9 ± 0.8*	8,1 ± 1,2	14.3 ± 1.4*	14.2 ± 1.2*	14.4 ± 1.1*
Acquired (n = 46)	1.2 ± 0.8	4.6 ± 0.7*	4.5 ± 0.8*	4.4 ± 0.7*	7.2 ± 0.9	14.0 ± 1.0*	14.1 ± 0.9*	14.0 ± 1.2*

Note. n – the number of eyes; * – $p < 0.05$ compared to the indicators before treatment.

Soft Inc., USA). The result was considered statistically significant when the probability of the type 1 error was $p < 0.05$.

RESULTS AND DISCUSSION

As a result of surgical treatment, elimination of blepharoptosis was achieved in all patients. In the early postoperative period, in 82.9 % of cases (68 eyes), lagophthalmos within 1–2 mm was noted, which was stopped within 1–2 months. The folds of the upper eyelids were pronounced and symmetrical.

Good result was achieved in 33 people (91.7 %) after surgical correction of congenital ptosis of the upper eyelid. In all cases, an increase in MRD1 indicators was noted in the postoperative period – on average up to 4.9 mm a year after surgery (Table 1). The width of the palpebral fissure increased to 10–12 mm, symmetrically to the healthy eye, the upper eyelid excursion improved to 14.3 mm (on average by 4–6 mm). The result was satisfactory in 3 (8.3 %) patients, which was due to the asymmetry of the palpebral fissure in comparison with the healthy eye. These patients underwent additional intervention to eliminate the asymmetry of the palpebral fissures in the late postoperative period.

A good result was achieved in 91.3 % of cases (42 eyes), satisfactory – in 8.7 % in the group of patients with acquired ptosis of the upper eyelid. MRD1 increased to an average of 4.4 mm after 12 months. The excursion of the upper eyelid was 14.0 mm, the width of the palpebral fissure increased to 11–12 mm and was the healthy eye. One patient was diagnosed with keratopathy due to a violation of compliance and non-compliance with the postoperative regime, which was stopped after a course of conservative therapy. One patient underwent repeated surgical intervention due to hypocorrection and asymmetry of the width of the palpebral fissure after the first surgery.

In both groups, there was an increase in levator function, an increase in the upper eyelid excursion to an average of 14.4 ± 1.1 mm in patients with congenital ptosis of the upper eyelid and up to 14.0 ± 1.2 mm in the group of patients with acquired blepharoptosis, the width of the palpebral fissure was symmetrical and amounted to 10–12 mm.

In all patients, the result remained stable throughout the observation period.

Performing dosed muscle-conjunctival resection of the upper eyelid cartilage in patients with partial upper eyelid ptosis and upper eyelid levator function of more than 5 mm allowed us to obtain a good postoperative result. This method is unlikely to be applied in patients with sharply reduced or absent function of the muscle that raises the upper eyelid, since this will not lead to the target result. When choosing a method of surgical treatment of patients with blepharoptosis, it is necessary to use a differentiated approach, and the assessment of levator function should be an integral part in the examination of patients with blepharoptosis.

CONCLUSION

The use of the method of dosed muscle-conjunctival resection of the upper eyelid cartilage in patients with partial ptosis of the upper eyelid and intact levator function made it possible to obtain a good functional and cosmetic result in congenital and acquired blepharoptosis in 91 % of cases.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest and any commercial or financial relations.

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PSYCHOLOGY AND PSYCHIATRY

ALEXITHYMIA AND PSYCHOSOMATIC DISEASES IN ADOLESCENTS: PRIMARY HEADACHES

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ABSTRACT

Background. *Alexithymia is traditionally regarded as a factor which influences the development of psychosomatic diseases and contribute to a more severe and prolonged course of somatic diseases. The high level of alexithymia indicates the deficit in cognitive processes associated with awareness, exteriorization and regulation of feelings and emotions. In recent years, a lot of research has been conducted on the comorbidity of alexithymia and psychosomatic diseases in adults, but there are very few studies in relation to children and adolescents.*

The aim. *To analyze psychosomatic diseases associated with the high level of alexithymia; in adolescents, to study the correspondence of alexithymia and central sensitization (CS) in adolescents with primary headaches (migraine and tension-type headache).*

Materials and methods. *The diagnosis of headache was based on the criteria for the International Classification of Headache, 3rd edition. The study group included 84 adolescents, average age – 14 [13; 16] (51 females, 33 males). CS was assessed using the Russian version of "Central Sensitization Inventory" (2020) for adolescents. Alexithymia was assessed using the Russian version of "Alexithymia questionnaire for children" (2019). Headache intensity was measured using the Visual Analogue Scale. There were also assessed the number of months and days per month with headaches; duration of night sleep; age of phrasal speech start.*

Results and discussion. *The results showed the direct correlation between levels of alexithymia and central sensitization ($rS = 0.49$; $p = 0.00001$), the number of days with headaches per month and central sensitization severity ($rS = 0.24$; $p = 0.027$). There was no significant correlation between alexithymia severity and headaches duration ($rS = 0.06$; $p = 0.5$), no reliable results on the correspondence of alexithymia severity, age of phrasal speech start and nocturnal sleep.*

Conclusion. *A high level of alexithymia is observed in adolescents with various somatic diseases. Primary headaches are associated with a high level of alexithymia and the severity of central sensitization. Pediatricians and neurologists should be advised to assess the level of alexithymia and central sensitization in adolescents with headaches.*

Key words*: alexithymia, adolescents, central sensitization, psychosomatics, comorbidity, Central sensitization inventory, Alexithymia questionnaire for children, primary headache*

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АЛЕКСИТИМИЯ И ПСИХОСОМАТИЧЕСКИЕ ЗАБОЛЕВАНИЯ У ПОДРОСТКОВ: ПЕРВИЧНЫЕ ГОЛОВНЫЕ БОЛИ

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РЕЗЮМЕ

Алекситимия традиционно рассматривается как фактор, предполагающий развитие психосоматических заболеваний, способствующий более тяжёлому течению соматических заболеваний. Высокий уровень алекситимии свидетельствует о дефиците когнитивных процессов, связанных с осознанием, экстериоризацией и регуляцией чувств и эмоций. Накоплено много данных в области коморбидности алекситимии и психосоматических заболеваний у взрослых, но при этом недостаточно исследований, посвящённых детям и подросткам.

***Цель исследования** – анализ психосоматических заболеваний, связываемых с высоким уровнем алекситимии у подростков; изучение корреляции уровня алекситимии и центральной сенситизации у подростков с первичными головными болями (мигрень и головная боль напряжения).*

***Материалы и методы.** Диагноз головной боли устанавливался на основании критериев Международной классификации головной боли 3-го издания. Группа исследования: 84 подростка, средний возраст – 14 [13; 16] лет (51 девушка, 33 юноши). Центральная сенситизация оценивалась с помощью русскоязычной версии «Опросника центральной сенситизации» (Central Sensitization Inventory). Уровень алекситимии оценивался с помощью русскоязычной версии «Опросника алекситимии для детей» (Alexithymia Questionnaire for Children). Интенсивность головной боли измерялась с помощью Визуальной аналоговой шкалы. Учитывались также количество месяцев и дней в месяц с головной болью; продолжительность ночного сна; возраст формирования фразовой речи.*

***Результаты и обсуждение.** Результаты показали прямую корреляционную связь между уровнем алекситимии и центральной сенситизации ($rS = 0,49$; $p = 0,00001$), количеством дней с головными болями в месяц и выраженностью центральной сенситизации ($rS = 0,24$; $p = 0,027$). Статистически значимой связи между выраженностью алекситимии, длительностью головных болей, дебютом фразовой речи и продолжительностью ночного сна не выявлено.*

***Заключение.** Высокий уровень алекситимии наблюдается у подростков с различными соматическими заболеваниями. Первичные головные боли сопряжены с высоким уровнем алекситимии и выраженностью центральной сенситизации. Врачам-педиатрам и неврологам целесообразно рекомендовать проводить оценку уровня алекситимии и центральной сенситизации у подростков с головными болями.*

***Ключевые слова:** алекситимия, подростки, центральная сенситизация, психосоматика, коморбидность, Опросник центральной сенситизации, Опросник алекситимии для детей, первичная головная боль*

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INTRODUCTION

Functional somatic symptoms (i.e., symptoms without a sufficient organic explanation) often manifest in childhood and adolescence and are very characteristic for this period of development. Their genesis is actively studied in modern literature. Alexithymia is considered one of the conditions that provoke the development of such symptoms. There is currently a sufficient number of studies on the correlation between a high level of alexithymia and the development of various psychosomatic diseases in adults. Specialists of different profiles describe the problem in an interdisciplinary way and receive more and more evidence that the deficit of exteriorization (including verbal) of emotions, sensations and feelings, which is observed at a high level of alexithymia, is associated with a higher probability of development and severe course of a variety of neurological, psychiatric, cardiovascular, bronchopulmonary, endocrine, etc. diseases, which can also be explained by the reduced ability of the alexithymic personality to develop coping strategies [1]. There is a lack of such data in relation to children and adolescents, while psychosomatic diseases often manifest themselves in these groups. The literature reports that the level of alexithymia is generally the highest in adolescence. According to O.S. Yutkina, only a third of adolescents aged 15–18 have a low level of alexithymia; almost 70 % of children belong to the borderline group or to the group with a high level of alexithymia. Moreover, girls aged 15–18 have a statistically significantly higher risk of alexithymia [2]. Difficulties in verbalizing sensations in adolescents are associated with a whole complex of both psychological and somatic factors. At a high level of alexithymia, these difficulties are noticeably aggravated, which affects the quality of diagnosis of diseases that require reliance on the verbal report of the patient (dizziness, pain, anxiety, depression). Alexithymic personalities tend to have problems at the level of choosing "inner words" by meaning and choosing the words themselves. Alexithymic patients either note that they do not even understand "inside themselves, without words" what exactly is happening, what sensations they experience, or assure that "inside themselves, without words" they distinguish one sensation from another, but it is too difficult to describe it. The feasibility of introducing visual support into the process of diagnosing diseases requiring a verbal report of the patient for alexithymic personalities is discussed. However, not all the subtleties of sensations, feelings and emotions can be adequately depicted paraverbally. In addition, the results of experiments on the mimic scale for pain determination in adults suggest that they are not suitable for alexithymic patients [3]. Studies on the assimilation of nonverbal communication show that it is difficult for people with a high level of alexithymia to adequately classify negative emotions expressed by nonverbal communication means, especially sadness, sorrow, despondency [4].

LITERATURE REVIEW

Russian research in the field of the study of alexithymia in children and adolescents is mainly concentrated around the following problems: alexithymia and emothional disorders, emotional intelligence problems [5]; diagnosis of alexithymia in children, including those with sensory disorders [6], prevention of alexithymia by psychological [7] and pedagogical methods: through the development of emotional intelligence [8], the application of art-therapeutic technologies [9]; psychological correction of alexithymia, including in adolescents with disabilities [10]; family alexithymia problems [11]. Part of the research is associated with the study of the correlation between alexithymia and bronchopulmonary diseases. In particular, N.L. Potapova et al. report that 76.6 % of children with bronchial asthma have a high level of alexithymia [12]. The correlation between the quality of life and the level of alexithymia in children with nephropathy was studied. It was revealed that children with nephropathy and a high level of alexithymia have statistically significantly lower level of quality of life than children with nephropathy and a low level of alexithymia [13]. However, it should be noted that 20-Item Toronto Alexithymia Scale (TAS-20; the Russian-language version for adults passed psychometric validation) [14] or 26-Item Toronto Alexithymia Scale (TAS-26; the Russian-language version for adults did not pass psychometric validation) [15] were mainly used in order to determine the level of alexithymia in most Russian studies, especially those conducted before 2015. These scales are not adapted for children and adolescents. Respectively, the main limitations of studies in which these tools were used are directly related to the alexithymia detection method itself, which may affect the reliability of the results.

Most foreign studies on child and adolescent alexithymia focus on child-parent relationships, parenting styles and features (overwhelming behavior of parents, emotional coldness, etc.); the correlation between a childhood trauma (emotional abuse or neglect), the level of alexithymia and the tendency for E-cigarettes use in adolescents is indicated [16]; the correlation between functional somatic symptoms in children with a high level of alexithymia and somatization in their parents [17] with low implicit self-esteem in children [18] is emphasized. However, a correlation between a school environment and the development of alexithymia in children has also been noted recently [19]. There is an increasing number of studies reflecting the connection between autistic traits and a high level of alexithymia not only in adults, but also in children and adolescents [20]. A combination of synesthesia, anxiety disorders and alexithymia in children has been reported [21]. A group of studies is devoted to the correlation of eating disorders and a high level of alexithymia [22]: it is revealed that obese and overweight adolescents have a higher level of alexithymia than adolescents with normal weight. A high level of alexithymia is noted in adolescents with epileptic seizures, psychogenic non-epileptic seizures and their combination [23].

Self-injurious behavior in children and adolescents with a high level of alexithymia is studied. It was revealed that alexithymia is a risk factor for non-suicidal self-injury (NSSI) in adolescents with depression [24], as well as with suicidal thoughts (but not attempts). Teenagers resort to self-injury because they cannot otherwise exteriorize their feel-

ings. Changes in the perception of somatic activation in children with a high level of alexithymia who have been subjected to abuse [25], the correlation between a high level of alexithymia and autoimmune diseases in children with experience of psychoemotional trauma [26] are also studied.

At the same time, it must be recognized that publications on the correlation of alexithymia and pain syndromes in children and adolescents are sporadic: possible correlations between alexithymia and fibromyalgia, alexithymia and headaches are revealed [27]. Again, the emphasis shifts more to the child-parent relationship (for example, it is revealed that alexithymic parents, as a rule, themselves have difficulties with coping strategies for overcoming pain, and, as a result, they cannot teach their children suffering from chronic headaches the correct coping mechanisms [28]).

Pain syndromes tend to become chronic, and it should be emphasized that alexithymia and central sensitization not only in adults, but also in adolescents, are the factors contributing to this chronicity, including in relation to headaches. Central sensitization (CS) is a phenomenon manifested by an increase in the responses of nociceptive neurons of the central nervous system in response to normal or subthreshold stimuli. As a result of the development of hypersensitivity of neurons of the central nervous system, a person begins to perceive various types of stimuli (pain, tactile, vestibular, etc.) more acutely, which in turn worsens the course of the underlying disease. Central sensitization can also manifest itself as sleep disturbance, unexplained muscle tension, soreness throughout the body and many other symptoms.

The aim of the study was to analyze psychosomatic diseases associated with a high level of alexithymia in adolescents, followed by the study of the correlation of the level of alexithymia and central sensitization in adolescents with primary headaches (migraine and tension-type headache).

MATERIALS AND METHODS

Alexithymia and central sensitization (CS) were assessed in adolescents with primary headaches (migraine and tension-type headache). The diagnosis of headache was based on the criteria for the International Classification of Headache, 3rd edition [29]. The inclusion criteria: age 13–18 years, presence of headaches (migraines or tension-type headaches), lack of treatment until the moment of treatment. The exclusion criteria: age under 13 and over 18 years, the presence of organic brain pathologies, cognitive and speech disorders. The study group included 84 adolescents, the average age was 14 [13; 16] years (51 girls, 33 boys). All participants of the study signed a voluntary informed consent. The study was approved at the meeting of the Local Ethics Committee of Kazan Federal University dated 28.03.2019.

The assessment of central sensitization was carried out using "Central Sensitization Inventory" (CSI) [30] for adolescents aged 14–17 years, which was translated from English and validated in Russian with the permission of one of the authors, Randy Neblett [31].

The assessment of alexithymia in adolescents was carried out using the Russian version of the "Alexithymia Questionnaire for Children" (AQC) [32], which is a simplified version of TAS-20. Translation into Russian and validation are carried out with the permission of the authors.

The intensity of the headache was measured using the Visual Analog Scale (VAS). The number of months with headache, the number of days with headache per month, the duration of night sleep, age of phrasal speech start were also taken into account (this factor must be taken into account due to the difficulty of verbalization of sensations in alexithymic personalities).

RESULTS AND DISCUSSION

The following parameters were evaluated in the study group (Table 1).

The results showed that there is a direct correlation between the level of alexithymia (the score on AQC) and the severity of central sensitization (the score on CSI) ($rS = 0.49$; $p = 0.00001$) (Fig. 1) in adolescents with primary headaches.

TABLE 1

AVERAGE VALUES OF STUDY PARAMETERS

Estimated parameters	Average value
Age	14 [13; 16]
Number of months with headaches	36 [12; 60]
Number of days per month with headaches	12 [6; 20]
Headache intensity (according to VAS)	60 [55; 80]
Score of alexithymia (according to AQC)	13 [8; 19]
Score of severity of central sensitization for adolescents (according to the CSI)	31 [24,5; 43]
Age of phrasal speech start (months)	18 [12; 24]
Duration of night sleep in hours	7 [6; 8]

It was revealed that there is no statistically significant correlation between headaches duration in months and alexithymia severity ($rS = 0.06$; $p = 0.5$). At the same time, there was a direct relationship between the number of days with headache per month and the severity of CS ($rS = 0.24$; $p = 0.027$) (Fig. 2).

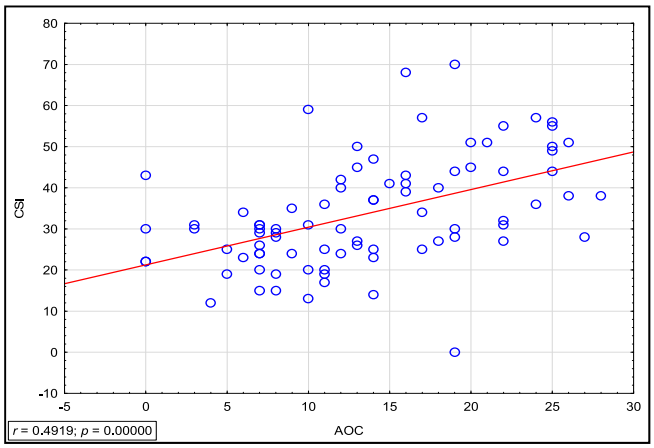

FIG. 1.
Correlation of "Alexithymia questionnaire for children" and "Central Sensitization Inventory" results

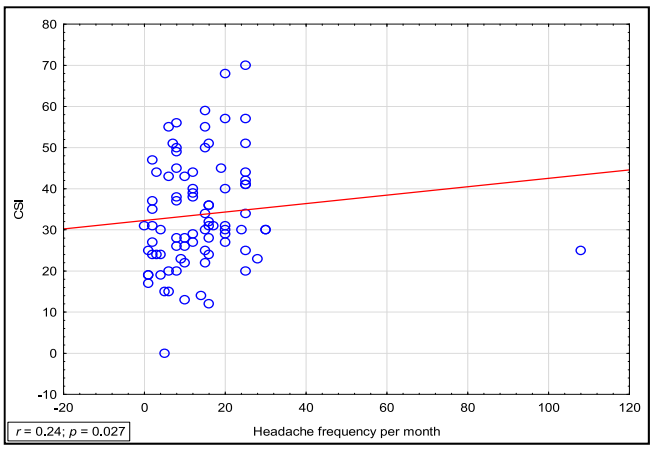

FIG. 2.
Correlation of the number of days with headache and the severity of central sensitization

There were also no statistically significant results of the correlation between alexithymia severity, level of central sensitization and headache intensity, as well as duration of night sleep and age of phrasal speech start. Data in the field of the correlation between sleep and alexithymia in foreign literature are contradictory; for example, there are: a) publications confirming that night sleep disorders occur because of a high level of alexithymia [33]; b) the hypothesis that alexithymia is a mediator between a decrease in sleep quality and child abuse (alexithymia occurs and then the quality of sleep decreases) [34]; c) data indicating that the formation of insomnia is influenced not by alexithymia, but by concomitant mental disorders [35].

In the course of further studies, it is necessary to assess the dynamics of the treatment in order to identify how high the level of alexithymia and central sensitization are prognostic factors for a more severe course and lower effectiveness of treatment of primary headaches.

CONCLUSION

Adolescence is multidimensional, transitional, complex period provoking both somatic diseases and behavioral characteristics. During adolescence, the risk of developing alexithymia sharply increases. Against its background, various somatic diseases (neurological, bronchopulmonary, etc.), as well as addictions, self-injurious behavior can occur. At the same time, somatic diseases and mental disorders themselves are risk factors for the development of secondary alexithymia, and a teenager may fall into a "vicious circle". It is indicative, in particular, that modern studies have begun to pay special attention to the prevention of the development of clinical conditions associated with difficulties in recognizing, expressing and regulating emotions, including alexithymia, which indicates the importance and prevalence of the problem.

Headaches in children and adolescents are one of the leading reasons to see a pediatrician or a neurologist. The results of the study show that primary headaches (migraine, tension-type headache) in adolescents are significantly associated with a high level of alexithymia and the severity of central sensitization. Thus, it is advisable to recommend assessing the level of alexithymia and central sensitization in adolescents with headaches during initial treatment, as well as during the therapy, in case of negative dynamics, to involve clinical psychologists in the management of the patient.

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Conflict of interest

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

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DIAGNOSIS OF PSYCHOGENIC (FUNCTIONAL) GAIT DISORDERS

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ABSTRACT

Psychogenic gait is common among patients with medically unexplained neurological symptoms and provides significant challenges to healthcare providers. Clinicians may arrive at a correct diagnosis earlier if distinctive positive signs are identified and acknowledged. Psychogenic disorders of posture and gait are common and are the major manifestation among 8–10 % of patients with psychogenic movement disorders. Psychogenic movement disorders can present with varied phenomenology that may resemble organic movement disorders. The diagnosis is based on clinical evaluation with a supporting history and classic features on neurologic examination. In functional gait disorders, walking is often bizarre and does not conform to any of the usual patterns observed with neurologic gait disorders. Astasia-abasia, an inability to stand (astasia) or walk (abasia) in the absence of other neurologic abnormalities, was the term applied by investigators in the mid to late 19th century to describe certain patients with a frankly functional gait. Other descriptive terms include gaits that resemble walking on ice, walking a sticky surface, walking through water (bringing to mind excessive slowness), tightrope walking, habitual limping, and bizarre, robotic, knock-kneed, trepidant, anxious, and cautious gaits. Ancillary testing, such as imaging and neurophysiologic studies, can provide supplementary information but is not necessary for diagnosis.

Key words: *psychogenic gait, neurological examination, gait analysis, positive signs*

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ДИАГНОСТИКА ПСИХОГЕННЫХ (ФУНКЦИОНАЛЬНЫХ) РАССТРОЙСТВ ПОХОДКИ

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РЕЗЮМЕ

Психогенные нарушения походки нередко встречаются у пациентов с неврологическими симптомами, которые необъяснимы с медицинской точки зрения. Психогенные расстройства позы и походки являются основным проявлением у 8–10 % пациентов с нарушениями психогенного движения. Они создают серьёзные диагностические проблемы для медицинских работников. Однако клиницисты могут прийти к правильному диагнозу уже при первичном осмотре, если они знают и умеют выявлять «позитивные» клинические признаки, направленные одновременно на исключение органической патологии и подтверждение психогенного (функционального) характера неврологических расстройств. При функциональных нарушениях походки ходьба часто бывает странной и не соответствует ни одному из обычных образцов, наблюдаемых при нарушениях неврологической походки. Они могут включать: астазию-абазию (неспособность стоять или ходить) при отсутствии неврологической патологии; нерентабельную походку на полусогнутых ногах; моноплегическую походку с подтаскиванием ноги, нередко с эквиноварусной установкой стопы; или напоминать «прогулку по льду», с небольшими осторожными шагами и фиксированной в голеностопном суставе лодыжкой. Вспомогательные обследования, такие как нейровизуализация и нейрофизиологические исследования, могут предоставлять дополнительную информацию, но не являются необходимыми для диагностики.

Ключевые слова: *психогенная походка, анализ походки, неврологический экзамен, позитивные признаки*

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Psychogenic (functional) movement disorders are quite common phenomena in clinical practice. 2–20 % of patients in specialized movement disorders clinics are diagnosed with functional movement disorders [1, 2]; 40 % of these patients have gait disorders without structural organic damage [3, 4]. In addition, among 30–60 % of patients who turn to a neurologist with gait disorders no organic pathology can be identified [5–8]. Psychogenic (functional) gait disorders (also called conversion disorders) are the loss of the ability to walk in the absence of neurological pathology. They are included in a wide range of functional neurological disorders (FNDs) and, in particular, in the category of movement disorders. In the foreign medical literature, when describing functional gait disorders, terms such as "psychogenic gait disorders" and "functional gait disorders" are equally used [9–11]; although in recent years the emphasis has been on the term "functional" – as less stigmatizing and offensive [12]. In this article we will use the term "psychogenic gait disorders" (PGDs).

PGDs can be isolated (e. g., astasia-abasia) or be part of mixed functional movement disorders: for example, in 6–8.5 % of patients, functional disorders of posture and gait are the main manifestation, and in 40 % – in combination with other functional movement disorders [3, 13–16]. Psychogenic astasia-abasia (or psychogenic dysbasia) is manifested by a disorder of movement coordination, which is characterized by the inability to stand or walk, despite the normal ability to move your legs in a sitting or lying position. Externally, the gait of such patients, especially in the doctor's office, has elements of drama and acrobatics (acrobatic gait). When walking, they sway in different directions, make sharp zigzag steps, tilt their torso forward (camptocormia) or lean back, cross their legs in a "hair-braiding" style. Some move on straightened and slightly separated legs (four-point gait), stumble over surrounding objects (which are usually soft), most often without falling; thus, on the contrary, demonstrating good motor and balance control. Sometimes patients with PGDs can actively try to fall when the doctor and/or family members are nearby; patients with organic diseases usually try to support themselves by looking for support nearby, and not crossing the room or corridor – as patients with PGDs do. Patients with psychogenic gait can walk normally if they think that no one is watching them. PGDs can be part of complex functional movement disorders, for example, with functional weakness or paresis in the form of hemiparesis, lower mono- or paraparesis; functional (psychogenic) parkinsonism or functional (psychogenic) dystonia. Therefore, some of them outwardly resemble hemiparetic, paraparetic and monoparetic gaits, others – dystonic or parkinsonian gaits. Gait disorders are often included in the structure of somatoform pain syndrome by the type of lumboishialgia with maladaptive pain behavior.

GENERAL APPROACHES TO THE DIAGNOSIS OF FUNCTIONAL NEUROLOGICAL DISORDERS

In the past, diagnosis of psychogenic or functional gait disorders was made after all potential organic causes were excluded. At the same time, the identification of "positive" symptoms, which make it possible to detect inconsistency of clinical neurological data with known diseases, is considered to be the most important in the diagnosis of functional neurological disorders. Modern diagnostic criteria for functional neurological disorders and, in particular, psychogenic gait disorders are based on DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, fifth edition, 2013) and ICD-10 (International Classification of Diseases. 10th Revision, 1992). In DSM-5, when making the diagnosis of "conversion disorder" (functional neurological disorder), the main emphasis was placed on clinical data, and less attention was given to concomitant psychological factors, as required in previous versions. Thus, neurological tests, according to the DSM-5, are an important step in reaching the diagnosis of a functional neurological disorder. They exclude organic disease and identify "positive" symptoms indicating a functional (conversion) disorder, which allows you to prevent unnecessary, and sometimes potentially dangerous, invasive studies that can support and consolidate functional neurological symptoms.

In ICD-10, conversion (dissociative) neurological abnormal movement disorders or weakness are included in section F44 (Dissociative disorders). Characteristic of this group of disorders are paresis and paralysis, astasia-abasia, etc. symptoms whose clinical manifestations do not correspond to the symptoms of known neurological disorders. ICD-11 (International Classification of Diseases for Mortality and Morbidity Statistics. 11th revision, 2020) defines functional gait disorders as follows: "dissociative neurological symptom disorder, with gait disturbance is characterized by symptoms involving the individual's ability or manner of walking, including ataxia and the inability to stand unaided, that are not consistent with a recognized disease of the nervous system, other mental, behavioral or neurodevelopmental disorder, or other medical condition and do not occur exclusively during another dissociative disorder" (code: 6B60.7 Dissociative neurological symptom disorder, with gait disorder). Thus, both DSM-5 and ICD-10 and ICD-11 point out an important feature – an inconsistency of psychogenic (functional) gait with known neurological or somatic disorders.

ANAMNESTIC AND CLINICAL FEATURES SUGGESTING THE PSYCHOGENIC (FUNCTIONAL) MOVEMENT DISORDERS

A. Anamnesis:

1. Symptoms most often occur acutely.
2. The course of the disease is stationary, without signs of progression.
3. Spontaneous remission or rapid regression of symptoms.
4. Concomitant signs of anxiety and depression are possible.
5. Multiple somatic complaints.

In some situations, a detailed psychosocial history allows you to identify the immediate cause of functional neurological disorders (FNDs). For example, it can be a job loss, divorce, sexual abuse, an insult or resentment. The disease can sometimes manifest itself after a minor physical injury, while abnormal movement disorders and pain sensations do not correspond to the degree of the injury [17]. Patients with conversion neurological disorders are often diagnosed with other unexplained psychosomatic manifestations, such as fibromyalgia, irritable bowel syndrome, atypical chest pain [18–20].

B. Clinical data:

1. Symptoms appear or increase when attention is drawn to them and decrease or disappear when the patient is distracted.

2. Functional neurological symptoms that are absent in the anamnesis may appear during the examination.

3. Such features of movements as amplitude, frequency, prevalence, are inconsistent and uncharacteristic of organic pathology features or signs.

Neurological examination allows to identify inconsistency of the obtained examination data with known symptoms or diseases; at the same time, it is important to distract the patient's attention during the examination. For example, a patient who cannot move their leg when asked to, begins to move it while putting on shoes or trousers after an examination. During neurological examination of patients with conversion neurological disorders, there are no objective signs of organic damage to the nervous system, for example: there are no pathological symptoms (Babinski sign), reflexive sphere without abnormalities, no muscle atrophy in the acute period. But at the same time, it should be taken into account that among some patients with FNDs, false neurological signs may be detected, such as hyperreflexia, Babinski pseudosymptom, pseudoataxia, etc. In functional hemiparesis, involvement of all anatomical structures on the "affected" half of the body may be noted (hearing loss, vision, hemiparesis and hemihypesthesia involving the face). In functional weakness in the arm or leg, the entire limb is involved, not individual muscle groups, and this may be accompanied by simultaneous muscle hypotension and hyperreflexia. During examination, non-anatomical sensitivity disorders may be detected (hypesthesia or anesthesia with a border strictly along the midline of the upper half of the trunk, or there is an "amputation character" of sensitive disorders involving the entire arm or leg). Patients with functional hemihypesthesia may claim that they do not feel vibration on one half of the sternum, although it is well transmitted through the bones.

C. Response to therapy. Lack of response to specific treatment aimed at a possible or suspected neurological disease, and a positive rapid therapeutic effect after psychotherapy or placebo.

D. Diseases associated with FND may include panic disorders, generalized anxiety disorders, post-traumatic stress disorders, dissociative disorders, social phobias or specific obsessive-compulsive disorders and personality disorders [5, 21, 22].

S. Fahn and P.J. Williams (1988) identified the following criteria for establishing FND: sudden or acute onset; spontaneous remissions or recurrence; inconsistency and variability of neurological symptoms during examination or dynamic observation that do not correspond to known neurological diseases; increased emphasis on painful manifestations; reduction or disappearance of certain functional symptoms when distraction or when using psychotherapy, suggestion or placebo; functional movement weakness or sensory (non-anatomical) disorders; pain sensations unexplained from the standpoint of modern medicine; excessive fear, startle response to an unexpected action; unnatural, bizarre movements; multiple somatization [23]. It should be noted that initially the authors proposed these criteria for the diagnosis of psychogenic dystonia; subsequently these diagnostic criteria have been extended to other FNDs.

DIAGNOSIS OF PSYCHOGENIC (FUNCTIONAL) GAIT DISORDERS

It should be noted that no gait pattern is pathognomonic for a functional gait disorder. For example, a bizarre gait can be observed in organic dystonia or chorea. In addition, functional and organic gait disorders can coexist in the same patient. Therefore, the diagnosis is often quite difficult, especially for an untrained neurologist. However, the diagnostic process should not be based on the exclusion of organic gait disorders, but on the search for positive clinical signs of functional gait disorders, namely: variability or incompatibility (i. e., variations in the clinical picture that cannot be reconciled with known organic pathology) and inconsistency (a combination of symptoms and signs that is not observed in organic lesions) [2]. For example, there is a sudden onset or rapid progression of gait disorders in the absence of injury or structural damage to the nervous or musculo-skeletal systems. Examples of inconsistencies in neurological examination may include the following: "scissor gait" in the absence of damage to the corticospinal pathway; antalgic gait in the absence of pain; bending of the knee (or knees) when walking with normal strength of the quadriceps muscle; significant improvement in gait with distraction.

DIAGNOSIS AND SUBTYPES OF PSYCHOGENIC GAIT DISORDERS

T. Lempert et al. (1991) distinguish six categories and six features of psychogenic gait [9]:

1) momentary fluctuations of stance and gait, often in response to a short suggestion;

2) excessive slowness or hesitation of locomotion incompatible with neurological disease;

3) "psychogenic Romberg test" manifested by a build-up of sway amplitude after a short delay and a decrease by distraction;

4) uneconomic postures with high wastage of muscular energy;

5) the "walking on ice" gait pattern, which is characterized by small cautious steps with fixed ankle joints;

6) sudden loss of stability and buckling of the knees, usually without falls.

Six suggestive features: 1) pseudoataxia: instability of posture and gait; 2) flailing of the arms; 3) dragging of the leg; 4) continuous flexion/extension of the toes; 5) bizarre tremor of the hands/legs/trunk/head; 6) sudden sidesteps.

PGDs can be of a diverse nature. Several characteristic subtypes of gait can be distinguished.

Leg-dragging gait (or monoplegic gait, with weakness, dragging and limping of one leg, while the other leg moves normally or almost normally) [16, 24, 25]. The patient drags a functionally weak leg behind the trunk, often with internal or external hip rotation and with inversion/eversion in the ankle. It is characteristic that the weak leg is dragged behind the patient from the hip and the front part of the foot is in contact with the floor ("as if it is magnetized to the ground"). Such patients use both hands to pull their leg up on the bed. Often there is a sudden weakness of the "affected" leg with bending of the knee joint, usually without falling. Positive Hoover test and other tests help to determine the functional weakness of the leg.

"Walking on ice" gait. This gait pattern is characterized by abnormal, excessively slow movement of both legs. The pattern of such a gait resembles walking on a slippery surface: cautious, slow, with reduced stride length and height, fixed knees and ankles; usually caused by simultaneous contraction of the agonist and antagonist muscles and outwardly resembling the gait during Parkinson's disease. Slow movements of the whole body are often noted, with a long time spent undressing and putting on clothes. In other situations, the hands may be pulled to the sides, which resembles walking on a tightrope. Gait can be improved by asking the patient to walk backwards, run or walk up and down stairs.

Truncal ataxia/instability. When walking, the patient sways from side to side, while often taking small side steps to avoid falling. Flailing of the arms. The flailing is observed only in the upper half of the body, whereas the legs move to correct the imbalance when the body vector is moved away from the body weight line; it seems that the person moves his or her legs in order not to fall and restore balance. Techniques and tasks aimed at distracting the patient improve postural instability, for example: guess the numbers written with a finger on their skin, solve complex arithmetic problems, count the months of the year in reverse order, etc. It is important to note that the balance of such patients during an objective examination is much better than they claim.

POSITIVE CLINICAL SIGNS THAT ALLOW TO IDENTIFY FUNCTIONAL WEAKNESS OF THE LOWER EXTREMITIES THAT AFFECT THE GAIT FUNCTION

Hoover test. The Hoover test has been used since 1908 to diagnose conversion paralysis during unilateral weakness of the lower limb. It was first described by the American physician Charles Franklin Hoover (1865–1927). This test has moderate sensitivity (63 %) and high specificity (100 %) [26–29]. This test is based on the phenomenon of coordination synkinesias. So, if a person lying on his or her on an examination table back lifts up one leg, then the heel of the other leg involuntarily exerts pressure on the table. It is difficult to use this test for bilateral weakness of the legs. The procedure: the doctor stands at the foot end of the bed, puts their hands under the patient's heels and asks him/her alternately to press his/her legs to the bed with maximum force. If the pressure of the patient's healthy leg on the doctor's hand is insignificant, then it can already be assumed that he or she is making little effort. Then the doctor asks the patient to lift the weak leg. In organic paresis, the doctor will feel an increase in the pressure of the healthy leg on the hand, i. e., the patient will try to support the paretic limb. Lifting a weak leg in functional paresis will not be accompanied by an increase in the pressure of the heel of the healthy leg on the doctor's arm (positive Hoover test). As an alternative, the patient is offered to lift a healthy leg up, while the doctor offer resistance to it, in which case the pressure of the heel of a functionally weak leg on the hand will increase and return to normal. This will not be observed with organic paresis, i. e. the paretic limb will exert weak pressure.

Sonoo abductor test. This test was developed by the Japanese neurologist M. Sonoo (2004) to detect unilateral inorganic paresis of the lower limb [30]. It is based, like the Hoover test, on the phenomenon of coordinating oppositional synkinesia and distraction. The procedure: the doctor stands at the foot end of the bed or the examination table with the patient lying on it and asks him/her to spread his/her legs apart as much as possible. Then the doctor puts his hands on the area of the lateral ankles of the patient's legs and, applying equal force, begins to shift them to the center. A weak leg, both in functional weakness and organic paresis, is detected immediately: it begins to move towards the healthy leg. Then the doctor moves the patient's healthy leg along the middle line and asks them to take turns moving their legs to the sides; at the same time, the patient's attention should be focused on the leg that is in motion. In psychogenic (functional) paresis, when the patient moves the healthy leg aside, focusing attention on it, the strength in the weak leg increases significantly and it is in a station ary state with equal external pressure from the doctor's hands. However, when the functionally weak leg is taken aside, the healthy leg also becomes "weakened", and it will shift towards the "pseudo-paralyzed" leg. In organic paresis, there will

be a different picture: when the patient takes the healthy leg to the side, it remains stationary (that is, it offers resistance), and the affected leg begins to move in its direction; if the patient move the paralyzed leg to the side, then the healthy leg continues to be in the initial (or stationary) state due to oppositional synergism. This test can also be performed in a sitting position on a chair or an examination table. In functional paresis, the strength in the hip will return to normal with the contralateral withdrawal of the healthy leg against the external resistance exerted by the doctor.

"Spinal Injuries Center" test. This test was described by I. Yugue et al. (2004) to assess psychogenic (conversion) paresis of the lower extremities [31]. The procedure and the evaluation method: the patient lies on their back, and the doctor passively raises their knees to a bent position, while their feet rest on the bed. Then the doctor removes his/her hands from their knees, and if the patient can keep their knees bent, the test is considered positive (that is, there are indications of psychogenic paresis). In organic paresis, the leg is not held in a bent position, and it will descend, bending the knee outward (in this case, the test is considered negative).

Muscle tone examination. A patient with psychogenic paresis may offer resistance to the doctor, which feels like an increase in muscle tone of the limb; but then suddenly there is no muscle resistance at all. However, the patient may resume resisting in the future (this sign is the phenomenon of "stepwise" weakness, "giveaway" or "compliant" weakness).

Lower Barre test. The patient is offered to lie on their stomach with their knees bent at right angles and hold them in this position. In organic paresis, the leg will quickly fall down with a possible oscillation of the leg. The leg of a patient with psychogenic paresis also quickly unbends, but without contraction of the hamstring muscles.

Chair test. This test helps to diagnose patients with functional gait disorder, demonstrating a significant discrepancy when walking in an upright position and when "walking" on a chair [32]. The procedure: the patient is first asked to "walk" back and forth to the examiner, while sitting in a revolving chair with wheels, at a distance of 6–9 meters. Then the patient is asked to walk the same distance without a chair. Patients with functional gait disorders will find it difficult to walk, but with the help of a revolving chair with wheels, they can usually move without problems. Patients with organic gait disorders have difficulty performing both tasks.

Huffing and Puffing sign. Patients with psychogenic gait disorders demonstrate excessive efforts when undressing, getting up from a chair, standing, and especially when walking, which are accompanied by frequent sighs after holding their breath, pained facial expressions, grunting, crying, extreme slowness, wiggling of the toes, sudden bending of the knees [33, 34].

Wartenberg test (test of the "game" of foot tendons). Normally, when a healthy person stands on one leg, the foot dorsiflexion muscles reflexively strain; in organic paresis, such tension of the tendons is weakly expressed or absent. Such alternating changes of muscle tendons in order to maintain balance are referred to as the "game" of tendons. The "game" of the tendons of the foot dorsiflexion muscles is detected among patients with psychogenic paresis of the foot (i. e., similar to a healthy person) when standing on one leg or a sharp push to the side.

For the purpose of differential diagnosis of PGD from organic gait disorders, other complicated tests are also used, for example, tandem walking; flank gait test; walking on heels, toes, with closed eyes, with rapid body turns; the Romberg test; running forward or backward. Gait assessment in a small doctor's office does not allow to detect all the relevant characteristics, so patients should also walk in a wide hallway or corridor.

The "psychogenic" version of the Romberg test: 1) a sharp deviation of the patient's body to or from the medical worker, but without falling to the floor; 2) excessive rocking of the body of a large amplitude after a delay of several seconds; 3) a significant improvement in standing in the Romberg pose with closed eyes when the patient is distracted (for example, with writing numbers on their back or solving arithmetic problems).

Flank gait test. The patient is offered to move sideways to the right and to the left. In organic hemiparesis, the patient moves better towards hemiparesis than in the opposite direction; and in psychogenic (functional) hemiparesis, walking sideways is impossible or significantly difficult, both in one direction and the other.

When testing for postural instability of patients with PG, uncharacteristic reactions are noted, unlike Parkinson's disease: for example, when a patient standing with his/her back to the doctor is pulled back by his/her shoulders (the retropulsion test), he/she takes sharp steps backwards, while flailing his/her arms, but does not fall [35] and sometimes even a simple pat on the shoulder can cause an abnormal anticipatory postural reaction [36].

Functional neurological disorders often occur acutely, mimic a stroke or organic lesions of the spinal cord (including with gait function disorder). Therefore, knowledge and mastery of the skills of "point-of-care" examination methods based on positive diagnostics allows doctors, especially those providing urgent or emergency medical care, to correctly diagnose, avoiding excessive economic costs for examinations and treatment [37–40]. It should be noted that none of the tests can be interpreted in isolation, but must be considered in the context of a complete clinical picture.

CASE HISTORY

Female patient I., 38 years old, after a number of psychotraumatic situations (a conflict at work, exacerbation

of her child's chronic disease) had a sudden speech disturbance, speech suddenly broke down, non-systemic dizziness, weakness in her left arm and leg. She was taken by ambulance to the neurological department of the hospital. After inpatient treatment she was discharged with the following diagnosis: "Ischemic stroke in the right middle cerebral artery. Arterial hypertension of 3rd degree, risk 4. Mild left-sided hemiparesis, hemihypesthesia. Transient dysarthria. Moderate vestibular-coordination disorders. Incapacity to work certification for 160 days, with different diagnoses: "vertebrogenic cervicobrachialgia, thoracalgia", fibromyalgia syndrome", "arterial hypertension", "polyosteoarthrosis". During the same period, she had several hospital admissions with different diagnoses. Due to long-term temporary incapacity for work, she was sent for a medical and social assessment (MSA).

Complaints and objective status during examination at the MSA Bureau. She entered the doctor's office on her own, in simple shoes, with a cane, leaning more on her right foot, walking extremely slowly, dragging her left foot in an equinovarus position. She makes numerous complaints: for almost constant headaches, pains throughout the body, a feeling of "goosebumps" on the body, lack of air, even at rest, limb weakness, more on the left side, periodic choking when eating due to the sensation of a foreign body in the throat ("like a lump stuck in the throat"), hoarse voice during agitation, fleeting deterioration of visual and auditory sensations; retrosternal pain, cardiac pain with irradiation to the left arm, scapula, occurring with any movement of the body, "I'm suffocating all the time, my heart hurts." Her husband helps her to dress and undress ("dizzy when tilting"). According to her, her husband and children do all the housework ("I can't even get dressed on my own"). Cranial nerves: pupils equal, pupillary responses are preserved, no oculomotor disorders, painless trigeminal points, well-functioning masticatory and mimic muscles, hearing is preserved, no nystagmus, soft palate arches mobile when phonating, tongue sticking out along the midline. Reflexes of oral automatism are negative. There is a periodic head tremor, which disappears when attention is diverted. The muscle tone in the right extremities has not changed, and the muscle tone in the left arm and leg changes according to the type of the phenomenon of "compliant" or "stepwise" weakness (i. e., periodically the patient resists the doctor when he/she examines the muscle tone, then suddenly ceases to exert muscle resistance). In the upper Barre test, the hands are at the same level, without pronation (i. e., there is no pyramidal weakness of any hand), although the left hand is clenched more loosely. Tendon reflexes from the hands of average vivacity, without significant difference. Knee and Achilles reflexes are triggered, with no convincing difference. Pathological hand and foot reflexes are not detected. Left-sided hemihypesthesia is determined strictly along the middle line. She performs coordination tests accurately, sways slightly in the Romberg pose, but does not change the pose. Positive Hoover test. No extrapyramidal disorders. No pelvic symptoms. In palpation the rectus muscles of the back are soft. No symptoms of tension of the nerve trunks.

Psychologist. The examination procedure is treated formally. There is no interest in the results of the examination. She learns the instructions to the tasks correctly, retains them in memory. She looks sad. She speaks in a low voice. The mood is low. Work pace is slightly low. Attention is draining. Performance is low. Mnestic functions are slightly low. Mnemogram 5-6-6-8. The efficiency of memorization is impaired. Delayed recall of 6 words. Arithmetic operations are performed at a moderate pace, without errors. When performing tasks aimed at the study of mental processes, an episodic decrease in the level of generalization is detected. From the side of thinking dynamics – slower pace. Graphic characteristics without signs of an organic nature. According to the test of Szondi, Luscher and the Beck Depression Inventory, it was revealed: the mood is low (17 points on the depression scale, which corresponds to moderate depression), increased sensitivity to external stimuli, decreased performance, difficulties in effective communication, the desire to flaunt oneself, increased anxiety, hypochondria. Personality has demonstrative traits. The internal model of the disease is inadequate. The rehabilitation potential of the disease is satisfactory.

Additional examination data. Ultrasound dopplerography (USDG) of the cervical vessels did not detect stenotic and occlusive lesions of arterial vessels. No focal pathology was detected during magnetic resonance imaging (MRI) of the brain. X-ray data of knee joints, hands, feet, ankle joints without abnormalities. ECG: sinus tachycardia. HR 100 bpm. Changes in the left ventricular myocardium with moderate diffuse abnormalities of repolarization processes. General analysis of blood and urine, biochemical blood tests – no abnormalities. Oculist examination – myopia of both eyes. Consultation with an endocrinologist – exogenous constitutive obesity of the 3rd degree.

The author's comment. Acute development of limb weakness and gait disorders can occur with stroke and functional (conversion) neurological disorder, which presents certain diagnostic difficulties. However, a thorough neurological examination with diagnostic clinical tests did not detect pyramidal symptoms that are observed in stroke. Thus, the patient has no pathological hand and foot signs (upper Rossolimo sign, Babinski sign), upper and lower Barre tests did not detect pyramidal weakness. Functional weakness in the leg was confirmed by the Hoover test. The muscle tone in the left extremities is changed according to the type of "compliant" or "stepwise" weakness. The gait is monoplegic – with weakness and dragging of the left leg behind the trunk with an equinovarus position of the foot. In a hemiparetic gait of organic origin, the paretic leg is straightened, and the foot, in the position of plantar flexion and supination, makes a semicircular movement

(circumduction) through the side. The sensitivity disorder is of a non-anatomical nature ("splitting" of sensitivity strictly along the middle line). Neuroimaging (brain MRI, USDG of the cervical vessels) and laboratory parameters (blood analysis, biochemical blood analysis) without abnormalities. It should also be remembered that the detection of any changes on the MRI may be of a residual nature, unrelated to the current disease. Therefore, paraclinical data must be compared with the clinical picture of the disease. Numerous psychosomatic complaints, comorbidity with mental disorders (anxiety, depression), secondary advantage (manipulation of family members) point in favor of FND. In addition, acute limb weakness, speech disorder (stuttering) occurred after traumatic situations. However, currently, according to the DSM-5, psychological stresses are not obligate when making a diagnosis of FND. The detection of "positive" physical signs and the performance of diagnostic clinical tests are crucial for this diagnosis.

Thus, it can be concluded that the patient has a "Functional (conversion) neurological disorder with movement and sensory disorders, gait disorders, chronic course."

It is important to note that about 8 % of cases of acute stroke mimics can be caused by functional neurological disorders [41, 42]. Neuroimaging (MRI, CT) is supported in these cases, but it does not confirm the diagnosis of a functional neurological disorder and does not exclude acute cerebral infarction with absolute certainty [43]. Therefore, clinical assessment at the point of care remains the best available method for distinguishing acute stroke and functional mimics of stroke.

CONCLUSION

Diagnosis of functional neurological disorders, including psychogenic gait disorders, should include a thorough clinical examination to identify positive signs. A "positive" diagnosis makes it possible to make a correct diagnosis at an early stage of the disease, and neuroimaging and neurophysiological studies can provide additional information.

Conflict of interest

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

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DENTISTRY

PREVALENCE OF MALOCCLUSIONS UNDER CONDITIONS OF PROLONGED INTRODUCTION OF SYSTEMIC FLUORIDES IN VARIABLE CONCENTRATIONS: LITERATURE REVIEW

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ABSTRACT

The pathogenesis of malocclusion, which are common among the population of all countries, is well represented in the professional literature. The occurrence of bite problems is associated with genetic and various environmental factors. Among the latter, fluorides which affect the prevalence of some dental diseases are of particular interest. However, there are few publications reflecting the frequency of malocclusion among the population in the regions with different levels of fluoride in drinking water. This problem seems to be significant in the context of the increasing impact of fluorine compounds on human health, including dental health.

The aim of the study. *To analyze the literature on the frequency of malocclusion among the population living in conditions of variable fluoride content in drinking water. A manual search of domestic and foreign literature was performed in the search databases PubMed, Medline and Google Scholar. From the initial list of publications, eighteen articles that met the inclusion criteria for the study were selected for analysis. We revealed significant variability of the research results. Some authors note a higher prevalence of malocclusions among the population under conditions of increased fluoride intake, others note a lower one, and still others did not reveal any differences between the values obtained in both samples. Most of the assessed publications did not methodologically meet modern international standards, and therefore were of little evidence.*

The literature data do not provide grounds for an unambiguous assessment of fluorine compounds as an environmental factor that indirectly affects the process of occlusion formation in humans and animals.

The review did not allow to make a definitive conclusion on the possible impact of systemic fluorides on the prevalence and pattern of malocclusion in humans and animals. It requires the implementation of studies that comply with the principles of evidence-based medicine.

Key words: *malocclusion, fluorides, drinking water, teeth, bone, teething, tooth size*

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РАСПРОСТРАНЁННОСТЬ ЗУБОЧЕЛЮСТНЫХ АНОМАЛИЙ В УСЛОВИЯХ ПРОДОЛЖИТЕЛЬНОГО ПОСТУПЛЕНИЯ ВАРИАТИВНЫХ КОНЦЕНТРАЦИЙ СИСТЕМНЫХ ФТОРИДОВ: ОБЗОР ЛИТЕРАТУРЫ

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РЕЗЮМЕ

Патогенез нарушений прикуса, широко распространённых среди населения всех стран, хорошо представлен в специальной литературе. Возникновение зубочелюстных аномалий связывают с генетическими и разнообразными факторами окружающей среды. Среди последних особый интерес представляют фториды, оказывающие влияние на распространённость ряда стоматологических заболеваний. Однако публикации, отражающие частоту малокклюзий среди населения в регионах с различным уровнем фторидов в питьевой воде, малочисленны. Данная проблема представляется значимой в условиях возрастающего воздействия соединений фтора на здоровье, в том числе стоматологическое, человека.

***Целью исследования** явился анализ литературы о частоте нарушений окклюзии у населения, проживающего в условиях вариативного содержания фторидов в питьевой воде. Выполнен ручной поиск отечественной и зарубежной литературы в поисковых базах PubMed, Medline, Google Scholar. Из первоначального списка публикаций для анализа выбраны 18 статей, отвечающих критериям включения в исследование.*

Выявлена значительная вариативность результатов исследований. Одни авторы отмечают более высокую распространённость зубочелюстных аномалий среди населения в условиях повышенного поступления фторидов, другие – меньшую, третьи не выявили различий между значениями, полученными в обеих выборках. Большинство оцениваемых публикаций методологически не соответствовали современным международным стандартам, в связи с чем были малодоказательными.

Данные литературы не дают оснований для однозначной оценки соединений фтора в качестве средового фактора, опосредованно влияющего на процесс формирования окклюзии у человека и животных.

Обзор не позволил сделать окончательного заключения о вероятном влиянии системных фторидов на распространённость и структуру малокклюзии у человека и животных. Требуется выполнение исследований, соответствующих принципам доказательной медицины.

***Ключевые слова:** зубочелюстные аномалии, фториды, питьевая вода, зубы, кость, прорезывание зубов, размеры зубов*

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RELEVANCE

Bite problems, or malocclusions, are one of the most common pathologies of the dental system in the world's child population [1–3]. Researchers pay special attention to the etiology of occlusion anomalies, since their prevention is impossible without identifying and eliminating the causes of the development of occlusal disorders [4]. In the majority of cases, bite problems are associated with uncontrollable (genetic predictors) and controllable (environmental factors) preconditions. Several authors point to a genetic background, the elimination of which is not currently possible, as the predominant precondition for the formation of occlusal pathology [5]. However, most specialists consider malocclusion to be unlikely as a result of a single cause, as the process of bite formation is long, with the probable influence of many negative factors that have a potentiating effect [6]. The identification and elimination of the latter is therefore of particular importance.

Despite a significant number of studies devoted to the etiology of malocclusion, there is not enough work on some factors that may play a role in the development of bite problems. Fluoride is widely distributed in the environment (water, soil, food, dental products, etc.). Sources of fluorides are varied, but most of them (up to 70 %) are ingested with drinking water. The trace element has a narrow "therapeutic corridor", and therefore its deficiency or excess can lead to changes in teeth and bones. Prolonged and excessive ingestion of fluoride compounds is accompanied by abnormalities in the functioning of various human and animal systems and organs, especially bone tissue. Fluorides affect bone remodeling processes, increase osteoblast proliferation and inhibit osteoclast function, and are an inducer of increased bone volume and mineralization [7]. Systemic fluorides, which have proven effects at the oral organs and tissues [8, 9], would be a potential cause of the formation of occlusal disorders among humans and animals [10]. Presumably prolonged exposure by fluoride compounds can act as a symbiotic factor (predisposing or preventing) for malocclusion.

In recent decades, as a result of the expansion of the range of sources of fluoride intake into the human body, there has been an increasing impact on the health of the population living in conditions of not only elevated but also optimal and even reduced levels of this trace element in drinking water. Currently, the main supplier of facultative fluoride to the child's body is fluoride-containing toothpastes, the systematic use of which during the formation of dental tissues in children from hydro fluorosis zones leads to the development or aggravation of the clinic of dental fluorosis [11–13]. The increasing prevalence of malocclusion and fluorosis among the child population in most regions of the world suggests the possible involvement of fluorides in the formation of bite problems, which justifies a review of previous studies in this area.

THE AIM OF THE STUDY

Analysis of the professional literature about the incidence of occlusion disorders in humans and animals under conditions of prolonged intake of systemic fluorides of different concentrations.

MATERIALS AND METHODS

A manual search of national and foreign literature was conducted in PubMed, Medline and Google Scholar search databases using keywords and their combinations: fluorides, drinking water, malocclusion, teeth, bone, teething, teeth size. Publication inclusion criteria for the review: taking into account the small number of thematic publications, both full-text and annotated articles containing at least data on the incoming fluoride levels and the prevalence/structure of bite problems were selected. Exclusion criteria: publications presented only with metadata (no annotation); publications that do not have information about fluorides. Only 18 of the 156 articles initially selected for review were published in Russia (13), Europe (1) and Asia (4) between 1959 and 2021. In view of the overall number and level of publications, we considered it possible to provide a detailed summary of the study in the section entitled "Study results".

STUDY RESULTS

The analysis of scientific articles confirmed the influence of fluorides on the body and health, including dental, of humans and animals [14, 15]. The effect of optimal and increased fluoride concentrations over the clinical and statistical picture of caries to temporary and permanent teeth has been proved. The prevalence and intensity of dental caries among children and adolescents from areas with low fluoride levels in drinking water were significantly higher than among peers living in hydro fluorosis areas [16, 17]. In a situation of excessive and prolonged intake, mainly from drinking water, of fluoride in the child's body during the period of dental formation, the probability of developing fluorosis increases [18]. There are studies indicating multidirectional, but in most cases negative effects of elevated fluoride doses on periodontal tissues [19, 20]. Studies on humans and animals have shown the negative impact of excess fluoride concentrations on bone cells and their remodeling processes, leading to chronic skeletal fluorosis, accompanied by bone lesions in the form of osteosclerosis or osteoporosis, degenerative changes of joints, calcification of ligaments, etc. [21].

The literature on the prevalence of malocclusions among the population in regions with different fluoride levels in drinking water is few and varied.

The first group of authors notes a lower prevalence of malocclusion among residents of areas with optimal and elevated fluoride levels in drinking water compared with the population from regions with low fluoride levels.

R.K. Aliyeva (1999) conducted an epidemiological survey of Azerbaijani children born and permanently residing in two regions that differ in the level of fluorides in water: in Baku (less than 0.5 ppm) and Absheron (more

than 1.5 ppm). The prevalence of bite problems in the child groups was 41 and 35 %, respectively, which allowed the author to consider the lack of fluoride in water as a risk factor for malocclusions among children [22].

An examination of children and adolescents 3–19 years old, born and permanently residing in Karaganda (Kazakhstan), using the unified methodology of the Central Research Institute of Dentistry and Maxillofacial Surgery (CRIDMS) revealed an inverse relationship between fluorosis and malocclusions. In a sample of children with a high prevalence of dental fluorosis, the incidence of dental caries and occlusion anomalies was significantly lower [23–25].

Y.L. Obraztsov (1994) studied the frequency of bite problems in samples of children with a higher and lower prevalence of dental fluorosis and showed that occlusal disorders were more frequently diagnosed among schoolchildren in the first group. In addition, the author points out that the content of fluoride affects not only the prevalence of occlusion disorders, but also the severity of their clinical manifestations [26]. Yu.L. Obraztsov and T.N. Yushmanova (2000), when studying trends in the incidence of malocclusions among children in the Arkhangelsk region over the past 15–20 years, revealed a significant increase (by 24 %) in their prevalence. The least frequent cases of DAA were observed among children living in an area with optimally elevated levels of fluoride in drinking water [27].

An analysis of dental morbidity among children in the Krasnodar region has revealed that low fluoride levels in natural drinking water sources contribute to the development of dental anomalies in children [28].

S.B.R. Chandra and colleagues examined 15-year-old Indian schoolchildren from regions with below optimal (less than 0.7 ppm), optimal (0.7–1.2 ppm) and above optimal (over 1.2 ppm) fluoride levels in drinking water using the dental aesthetic index (DAI). The prevalence of malocclusions was significantly higher among the pupils in the first group. The average DAI score in the first group of pupils was statistically significantly higher than in groups two and three, i. e. it decreased with increasing fluoride concentration in drinking water. Severe and very severe bite abnormalities were more common in the first group than in adolescents in the other groups [29].

However, **the second group of authors** found a higher prevalence of occlusal abnormalities among children and adolescents who consumed water with a higher fluoride content.

A large-scale study to assess the dental morbidity of the population of different climatic and geographical regions of Russia has confirmed that the prevalence of dental abnormalities was significantly higher among residents of areas with increased fluoride content in water supply sources compared with the residents of areas characterized by deficiency of this micronutrient [30].

Based on a review of the literature that evaluated the risk factors for the development of bite problems among children, the lowest prevalence of anomalies was found in areas with optimal fluoride levels in drinking water, while the highest prevalence was found in areas with high fluoride levels [31].

A study of the dental status among children living in the emission zone of the Tajik aluminium factory and children from pollution-free area showed a higher prevalence of bite problems among children from the first group (39.8 %) compared to their peers from the second group (3.7 %) [32].

The unfavorable geochemical situation in Transbaikalia (elevated fluoride content in drinking water) has led to a high incidence of malocclusion, fluorosis and dental caries. In the structure of bite abnormalities among children under three years old, a prognathic bite combined with an open bite, as well as an open bite, were observed to be statistically significantly more prevalent than in areas with insufficient and optimum fluoride levels in the water. The inconsistent and early eruption of temporary teeth among these children is a risk factor for the formation of malocclusions. In areas of hydro fluorosis, children are 2.2 times more likely to develop dental pathological abnormalities, including the DAA [33–35].

A third group of authors found no or equivocal in their interpretation about the association between the clinical and statistical characteristics of malocclusion among children and adolescents and the level of incoming fluoride.

V.V. Belyaev and colleagues performed a single-stage dental examination among 361 schoolchildren aged 12 and 15 years from Tver, according to the methodology of the World Health Organization (1997), who lived in conditions of optimally elevated fluoride levels in drinking water (1.5–4.5 ppm). A high prevalence of dental fluorosis and dentoalveolar anomalies has been found among the pupils examined. There were no statistically significant differences between the prevalence of bite problems, most DAI components in samples of pupils with and without dental fluorosis of varying severity. An increase in the incidence of anteroposterior ratio deviations of the first permanent molars has been observed in groups of pupils with severe dental fluorosis [36, 37].

Z. Krzoglu et al. surveyed two samples comprising a total of 332 Turkish preschool children (3–6 years) permanently living in regions with elevated (mean 2.16 ppm) and low (0.04 ppm) fluoride levels in drinking water. The socio-economic status of children in both groups was comparable. Other possible risk factors were assessed using questionnaires. There is variability in the frequency of various occlusal abnormalities between the groups. In only half of the cases were the differences between the values obtained statistically significant. An anterior cross-bite appeared to be statistically significantly more common in children in the first group, an anterior open bite and incisor crowding in children in the second group [38].

A. Masztalerz et al. examined a total of 372 children aged 12 in four areas with varying fluoride concentrations in drinking water and air. The severity of pathological occlusion was assessed using the Eismann – Masztalerz method. Optimal concentrations of fluorides in drinking water (0.7–0.9 ppm) have been demonstrated to reduce the severity of abnormalities apart from crowding of teeth, whereas concentrations above the optimum (4.0–7.0 ppm) as well as fluoride-contaminated air are among the causes of incisor crowding [39].

DISCUSSION

This review of the assessment of the prevalence and severity of pathological occlusion in the population under conditions of different levels of fluoride intake allowed us to identify three main groups of studies characterized by contradictory results.

The findings of the first group about the higher incidence of malocclusions among child populations from regions with low fluoride levels in drinking water seem logical. In such populations, dental caries is more common, and its intensity becomes higher. The probability of developing caries complications in the form of pulp and periodontal inflammation, early extraction of temporary teeth and first permanent molars increases. Generally, the resulting defects cannot be repaired with removable partial dentures or fixed space maintainers, which results in displacement of neighboring teeth and the formation of bite problems [40, 41].

This algorithm for maxillofacial disorders is particularly important in regions with insufficient and low levels of dental care available to children. The model under consideration is not unambiguous in the long term, but it is recognized as relevant for the age at which the majority of malocclusionsand deformities are formed [42].

With multiple caries, especially in conditions of hypophthorosis, the intensity of carious lesions increases not only of the teeth, but also of their surfaces, mainly those in contact [43]. Given the lack of accessibility of pediatric dental care in some regions of the world, multiple carious lesions of the proximal surfaces of teeth in children can lead to a reduction in the mesiodistal dimensions of the crowns, sagittal drift of the affected teeth, shortening of the dental arches and eventually to secondary dental displacement and impaired occlusion [44–46]. Asymmetric mastication, pathological reorientation of the occlusal contacts, often occurring against a background of secondary adentia, can be a trigger for the formation of occlusal disorders, especially in the presence of additional risk factors. In particular, an unilateral mastication could cause an unilateral posterior crossbite, which is a broad asymmetrical bite anomaly characterized by an inverse relationship between the upper and lower vestibular dental cusps in the area of the molars and premolars on the same side of the dental arch. Patients with a unilateral posterior crossbite have altered mastication cycles and the masseter muscle is less active on the side of the crossbite than on the contralateral side [47].

The aforementioned findings demonstrate the benefits of administering optimal doses of fluoride compounds from natural or fluoridated drinking water sources to children, associated with the control of both dental caries and associated dental diseases and conditions, including bite disorders [7]. However, there are arguments for controversy regarding this point of view. A number of authors have reasonably observed that studies of the effects of fluorides applied to human and animal oral organs and tissues without having taken into account all possible risk factors can lead to misinterpretations of the results obtained [48, 49]. An underestimation of a significant variable, such as the socio-economic status of a family or individual, contributes to a misinterpretation of the information obtained in the study. As a rule, individuals with lower status are typically associated with inadequate dental compliance (do not regularly visit the dentist for the prevention and treatment of dental diseases, do not follow the generally accepted recommendations for individual oral hygiene, etc.), do not have the opportunity to eat rationally, etc., and are more likely to have untreated dental caries and its complications. Among such individuals, even in conditions of optimal and increased fluoride intake, available level of dental care, untreated dental caries and its complications, cases of tooth extraction without subsequent dental prosthetics are more often revealed [50, 51].

The studies underline the fact that the range of human and animal sources of fluoride has expanded in recent decades, significantly altering the epidemiology of fluoride-associated dental diseases directly or indirectly. The current literature data suggest that the algorithm of the variables "dental caries – dental defects – maxillofacial anomalies" that has been formed in the past decades remains relevant, and the order of "systemic fluorides – dental caries – dental defects – maxillofacial anomalies" now needs further study and possibly reinterpretation.

The second group of studies analyzed by us indicates a higher incidence of bite problems among the population consuming water with optimal or elevated fluoride levels, which can also be explained.

Chronic fluoride intoxication is accompanied by abnormalities in the functioning of cardiorespiratory, neuroendocrine, musculoskeletal, dental and other human systems and organs. The positive and negative effects of fluoride on bone tissue have been described in the literature [52]. Fluoride has been demonstrated to increase the proliferation of osteoblasts and inhibit osteoclast function, and is an inducer of increased bone volume and mineralization [53]. A significant number of factors affecting bone metabolism and the complexity as well as diversity of the manifestations of fluoride effects in bones, including jaw bones, which go beyond just changes in bone density, have been pointed out [54]. The results of studies examining the rate of tooth movement under the influence of orthodontic appliances in humans and animals under prolonged systemic ingestion of a variety of fluoride concentrations have confirmed these findings [55].

Orthodontic treatment is associated with the movement of teeth through the reconstruction of the alveolar bone. The pressure exerted on the crown of the tooth is transmitted through the root to the ligamentous apparatus of the periodontium and the alveolar bone. In the tissues of the jaw bone, compression zones arise, where the alveolar bone is resorbed, and stretching zones, where the bone is formed. It has been shown that different concentrations of endogenous fluorides can have several different effects on orthodontic tooth movement and in combination with mechanical forces and associated factors can have synergistic, preservative or inhibitory effects [56]. Intensive systemic use of sodium fluoride in order to prevent caries during orthodontic treatment can slow down the rate of tooth move-

ment and prolong the period of active treatment [57]. Animal studies have shown that fluorides, especially with long-term exposure, reduce the rate of tooth movement while undergoing simulated orthodontic treatment [58]. Along with medications (bisphosphonates, corticosteroids, estrogens, aspirin, diclofenac, ibuprofen, indomethacin, etc.), endogenous fluorides can be classified as factors that slow down tooth movement during orthodontic treatment [59]. However, a number of authors do not share this opinion. During orthodontic treatment of young people from cities with very low (0.05 ppm) and elevated (2 ppm) fluoride levels in drinking water, a higher rate of tooth movement was detected among the second group of patients [60]. Y.U. Yangyang et al. (2016) among adolescents without fluorosis and with dental fluorosis of varying severity showed significantly greater tooth movement distance and less alveolar bone resorption area in the group of patients with fluorosis at every treatment stage. The authors conclude that fluorides play a positive role in bone remodeling during orthodontic treatment [55]. However, simulated orthodontic treatment of animals confirmed a statistically significant increase in the number of osteoblasts on the extension side and a decrease in their number on the compression side, but showed no difference in the dynamics of tooth movement under conditions of insufficient and excessive systemic fluoride intake [61]. The variability in the results of these studies may be associated with limitations in their design, as bone remodeling is a multifaceted and complex process, influenced by a number of different factors, including genetics and environmental conditions [62].

Tooth eruption is a genetically determined process, but is influenced by a number of general and local factors: racial, ethnic, gender, socio–economic, geographical and others [63]. Animal studies have confirmed a delay in the eruption of mandibular molars in experimental rats that constantly consumed fluoridated water [64], which allows us to consider fluoride as a potential environmental factor not only prolonging the timing of tooth emergence in the mouth, but also indirectly negatively influencing the formation of occlusion.

The morphology of the teeth, especially the mesiodistal and buccolingual dimensions, has a significant influence on their position in the dentition and the development of occlusion in the temporary and permanent bites. In turn, tooth size is influenced by both genetics and numerous environmental factors [65]. Among the latter are fluorides, the prolonged and excessive intake of which, mainly with drinking water, is capable of affecting the size and morphology of teeth in humans and laboratory animals. This effect has been noted in numerous studies and has been confirmed by the work of the Turkish authors, who in a study of dental parameters among children and adolescents with fluorosis revealed smaller crown sizes in the permanent upper incisors, second premolars and first molars. The sizes of temporary teeth with and without fluorosis did not differ [66]. Similar findings were observed by their compatriots, who revealed that the mesio-distal dimensions of teeth were larger among adolescents without fluorosis and with a normal bite than among their peers with this pathology. However, in most cases there were no statistically significant differences between the indicators obtained in the compared groups of patients [67]. The significantly smaller diameter and height of the cusps of fluorotic permanent teeth is indicated by C.J.M. Ten et al. [68]. Y.B. Aswini and colleagues, analysing the literature findings about the influence of fluoride on dental morphology, pointed to less pronounced (small and wide) fissures in molars among patients who consumed water or products with elevated fluoride concentrations [69]. Ameloblasts are known to be highly sensitive to internal and external influences. Indonesian scientists have demonstrated a negative effect of high doses of fluoride in drinking water against enamel development in rats as a result of apoptosis in ameloblasts and an increase in intercellular space, resulting in the formation of thinner enamel and smaller teeth [70].

The analysis of scientific studies carried out at different times in different countries, including different age and social groups of the population, as well as animals, did not allow an unequivocal assessment of the impact of systemic fluorides on bone tissue, teething processes and their macromorphology. However, obtaining the most objective information possible about the issue is of great importance in clinical dental practice. The morphological characteristics of the teeth are the most important factors influencing the positioning of the teeth in the dental rows [71], whose abnormal size correspondence leads to anomalies of occlusion in different directions, changes in function [72].

The literature review allowed us to note two main points. First, despite hundreds of studies conducted over the past decades to assess the impact of systemic fluorides on human dental health, only eighteen publications were accepted for analysis in accordance with the selection criteria for this study, demonstrating the relevance of the problem in question. Second, the methodological basis of the majority of studies published more than a decade ago varies considerably and is characterized by the variety of assessment tools used by the authors (different classifications, indices), racial and age samples of the surveyed population, levels of professional competence of experts, which served as one of the reasons for the inconsistency of the results obtained. The use of unified methodological approaches in similar case studies is known to enable comparison of results at national and international levels and to increase the reliability of the results [73]. Even significant quantitative indicators without proper research quality are unable to influence the outcome of the review. Thus, the conclusion of a recent critical review assessing the effect of systemic fluorides against statistical indicators of dental caries, a topic that is very widely and extensively represented in the specialized literature, pointed out the lack of studies that meet the criteria for inclusion in the Cochrane review and the need for further in-depth studies [74].

CONCLUSION

A review of the literature demonstrated the ambiguity of data on the frequency and structure of malocclusions un-

der conditions of long-term variable intake of systemic fluoride compounds, i. e. its possible role in the formation of human and animal occlusion. Although a summary of a review based on an analysis of publications in the Google, Rutgers Library, PubMed, and Medline databases did not identify fluoride as a risk factor for bite problems [75], the findings of this study justify further research in this area, with qualitative studies based on evidence-based methodologies.

Conflict of interest

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

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SURGERY

VACUUM-ASSISTED LAPAROSTOMY IN SEVERE ABDOMINAL TRAUMA AND URGENT ABDOMINAL PATHOLOGY WITH COMPARTMENT SYNDROME, PERITONITIS AND SEPSIS: COMPARISON WITH OTHER OPTIONS FOR MULTISTAGE SURGICAL TREATMENT (SYSTEMATIC REVIEW AND META-ANALYSIS)

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ABSTRACT

Background. *The concept of multistage surgical treatment of patients has been established in surgery rather recently and therefore the discussions on the expediency of using a particular surgical technique in a specific situation still continue. Vacuum-assisted laparostomy is being widely implemented into clinical practice for the treatment of abdominal compartment syndrome, severe peritonitis and abdominal trauma, but the indications and advantages of this method are not clearly defined yet.*

The aim. *To conduct a systematic review and meta-analysis on the comparison of the effectiveness of vacuum-assisted laparostomy with various variants of relaparotomy and laparostomy without negative pressure therapy in the treatment of patients with urgent abdominal pathology and abdominal trauma complicated by widespread peritonitis, sepsis or compartment syndrome.*

Material and methods. *A systematic literature search was conducted in accordance with the recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analyses. We carried out the analysis of non-randomized (since January 2007 until August 6, 2022) and randomized (without time limits for the start of the study and until August 6, 2022) studies from the electronic databases eLibrary, PubMed, Cochrane Library, Science Direct, Google Scholar Search, Mendeley.*

Results. *Vacuum-assisted laparostomy causes statistically significant shortening of the time of treatment of patients in the ICU and in hospital and a decrease in postoperative mortality compared to other variants of laparostomy without vacuum assistance.*

Conclusion. *To obtain data of a higher level of evidence and higher grade of recommendations, it is necessary to further conduct systematic reviews and meta-analyses based on randomized clinical studies.*

Key words: *vacuum-assisted laparostomy, laparostomy, vacuum therapy, negative pressure therapy, open abdomen, on-demand relaparotomy, planned relaparotomy, abdominal compartment syndrome*

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ВАКУУМ-АССИСТИРОВАННАЯ ЛАПАРОСТОМИЯ ПРИ ТЯЖЁЛОЙ ТРАВМЕ ЖИВОТА И УРГЕНТНОЙ АБДОМИНАЛЬНОЙ ПАТОЛОГИИ С КОМПАРТМЕНТ-СИНДРОМОМ, ПЕРИТОНИТОМ И СЕПСИСОМ: СРАВНИТЕЛЬНЫЕ АСПЕКТЫ С ДРУГИМИ ВАРИАНТАМИ МНОГОЭТАПНОГО ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ (СИСТЕМАТИЧЕСКИЙ ОБЗОР И МЕТААНАЛИЗ)

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РЕЗЮМЕ

Обоснование. *Концепция многоэтапного хирургического лечения больных утвердилась в хирургии относительно недавно, поэтому продолжаются дискуссии о целесообразности использования той или иной методики в конкретной ситуации. В клиническую практику лечения абдоминального компартмент-синдрома, тяжёлого перитонита и травмы живота всё шире внедряется вакуум-ассистированная лапаростомия, но пока недостаточно чётко определены показания и преимущества данного метода.*

Цель исследования. *Проведение систематического обзора и метаанализа по сравнительной оценке эффективности вакуум-ассистированной лапаростомии с различными вариантами релапаротомий и лапаростомий без терапии отрицательным давлением при лечении пациентов с ургентной абдоминальной патологией и повреждениями живота, осложнёнными распространённым перитонитом, сепсисом или компартмент-синдромом.*

Материал и методы. *Систематический поиск литературы проведён в соответствии с рекомендациями Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Произведён анализ нерандомизированных (с января 2007 г. по 6 августа 2022 г.) и рандомизированных (без временных ограничений начала исследования по 6 августа 2022 г.) исследований из электронных баз eLibrary, PubMed, Cochrane Library, Science Direct, Google Scholar Search, Mendeley.*

Результаты. *Вакуум-ассистированная лапаростомия приводит к статистически значимому сокращению сроков нахождения пациентов в отделении реанимации и интенсивной терапии и в стационаре, снижению послеоперационной летальности в сравнении с другими вариантами лапаростомий без вакуума.*

Заключение. *Для получения данных более высокого уровня достоверности доказательств и убедительности рекомендаций необходимо дальнейшее проведение систематических обзоров и метаанализов на основе рандомизированных клинических исследований.*

Ключевые слова: *вакуум-ассистированная лапаростомия, лапаростомия, вакуумная терапия, терапия отрицательным давлением, «открытый живот», релапаротомия «по требованию», программированная релапаротомия, синдром интраабдоминальной гипертензии, абдоминальный компартмент-синдром*

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INTRODUCTION

"When does a surgeon who is not a novice worry?
Not at the time of surgeries...
The worries of the surgeon begin after surgery,
when for some reason a high fever persists
or the abdomen does not subside, and now, on the tail of the missed time,
one must mentally cut open, see and understand
how to correct the mistake without using a knife.
It's useless to blame a postoperative complication
on a random side cause."
A.I. Solzhenitsyn, 1967

One of the most difficult problems of surgery remains the treatment of abdominal pyoinflammatory diseases and complications. In the structure of surgical morbidity, peritonitis and its destructive lesions of the abdominal cavity are among the most important [1]. The number of patients with infected forms of pancreatic necrosis, gastrointestinal perforation, traumatic injuries of the abdominal cavity and retroperitoneum, widespread peritonitis of various etiologies increase [1]. At the same time the mortality rate does not have any tendency to decrease and ranges from 19 to 70 **%** with the development of septic shock [1–6].

Saving the patient's life in these cases depends on timely diagnosis and effective surgical intervention. In extremely severe and hemodynamically unstable patients with widespread peritonitis, abdominal trauma in the presence of compartment syndrome, peritonitis, sepsis, there are currently three methods of multistage surgical treatment after primary laparotomy within the framework of *source control* and *damage control* strategies [1, 7–13]:

- on-demand relaparotomy (emergency);
- programmed relaparotomies (planned);
- various variants of laparostomy ("open abdomen" technique).

Current knowledge confirms the concept according to which on-demand relaparotomy performed with clinical deterioration of the patient's condition or lack of improvement is an effective measure to eliminate permanent or repeated infection of the abdominal cavity [1, 12, 14]. When comparing the results of treatment of patients with "open abdomen" and on-demand relaparotomy in a randomized clinical trial by F.A. Robledo et al. (2007) [15], the mortality was significantly higher in the group of patients with open abdominal management – 55 **%** versus 30 **%**, respectively, but no statistical significance of differences was obtained (cit. according to [12]). In a randomized study of O. van Ruler et al. (2007) [16], advantages of on-demand relaparotomy over planned lavages were shown in terms of reducing the cost of patient treatment, the duration of hospital stay, and the duration of the recovery period, but also no statistically significant difference in mortality was obtained (cit. according to [7, 12, 17]). Difficulties of timely diagnosis of postoperative intraabdominal complications and the resulting delays in repeated intervention determine still high frequency of poor treatment results when choosing a closed abdominal management method [1, 2, 4, 7, 12]. Most of the currently existing integral scales (scores) for assessing the severity of peritonitis and abdominal sepsis are not effective for determining indications for emergency relaparotomy [4].

Programmed relaparotomy is used when it is impossible to eliminate or reliably delimit the source of widespread fibrinous-purulent or fecal peritonitis and complete lavage of the abdominal cavity in a patient with septic shock, in the presence of intra-abdominal hypertension syndromes (IAHS; syn. abdominal compartment syndrome) and intestinal, doubts about the viability of the intestine and the need to perform a delayed intra-abdominal anastomosis [1, 2, 4, 7, 10, 12, 18]. The most obvious disadvantage of such a patient management tactic is multiple mechanical traumatization of abdominal organs during reoperations, in some cases leading to serious complications: suppuration of postoperative wounds, complete eventration, and the formation of intestinal fistulas; among patients with tertiary peritonitis, it can contribute to the progression of Multiple Organ Dysfunction Syndrome (MODS) [4, 7, 13].

The occurrance of laparostomy (*laparo* (Lat.) – abdominal wall; *stomia* (Lat.) – the operation of applying an artificial external fistula, stoma) is associated with Johann Mikulich-Radetsky, who in 1884 proposed tamponing the abdominal cavity with iodoform gauze with an untreated laparotomy wound in order to remove exudate from the abdominal cavity and delimit the purulent process [19]. This method is possible in two conditional variants – open and closed technology; using the latter, the wound of the abdominal wall is closed with temporary devices [18]. Laparostomy formation in the classic "open" option is of limited use in situations when visceral edema prevents effective closure of the abdominal cavity with its own tissues. Abdomen temporary closure is possible with the help of adhesive membranes, synthetic and biological meshes [20], vacuum (VAC, vacuum-assisted closure) therapy with negative pressure in the abdominal cavity and the laparostomy formation using negative pressure wound therapy (NPWT) [1, 7, 9, 18, 21]. VAC-laparostomy implies the mandatory presence of a protective (more often – perforated polypropylene) film, a polyurethane sponge, a sealing film, and a device for constant vacuum aspiration. Air is evacuated from the abdominal cavity through a system of tubes with a special vacuum generator, resulting in negative pressure, which accelerates the formation of granulation tissue, improves blood supply, reduces contamination of the abdominal cavity, localizes and reduces the exposure time of pathological peritoneal exudate [2, 4, 8, 12, 22–24].

Initially, VAC-laparostomy was used differently, in particular in the USA and Latin America, as well as in South-East Asia, it was indicated mainly in severe abdominal trauma, while in the UK, Germany and our country – mainly in widespread fibrinous-purulent/fecal peritonitis and sepsis [19, 25]. The advantage of this method is the elimination of IAHS, which contributes to the normalization of the respiratory, cardiovascular and nervous systems [1, 2, 7, 25, 26]. But even here discussions arise. On the one hand,

the use of NPWT solves the problem of increased intra-abdominal pressure and the development of IAHS, reduces the risk of severe abdominal sepsis in fibrinous-purulent/fecal peritonitis, leads to a decrease in mortality, the duration of hospital stay and the cost of treatment [7, 12, 23–25, 27]. Most researchers note a statistically significant improvement in the results of primary muscle-aponeurotic closure in comparison with other methods of laparostomy management [7, 12, 25]. At the same time, exposure to negative pressure can lead to ischemia of intestinal areas, development of petechial and erosive bleeding and increases the risk of intestinal fistulas [1, 4, 5, 7, 10, 12] from 5 to 20 % of observations [25, 27].

In 2015, the World Society of Emergency Surgery and the Panamerican Trauma Society initiated the creation of the International Register of Open Abdomen (IROA), and in 2017 the first results of an international study were published, according to which the VAC technique, compared with other types of laparostomy, has the lowest mortality and complications rates [9], but the risk of fistula formation is among the leaders along with the Wittmann patch (13.5 and 17.6 %, respectively), yielding the latter "leadership" [7, 28, 29].

There is a large number of studies in the modern press comparing various variants of laparostomy with vacuum therapy with each other (more recent publications), on-demand relaparotomy with programmed relaparotomy (older publications), but there are few studies comparing vacuum laparostomy with different variants of relaparotomy and laparostomy without negative pressure therapy, while these very methods remain relevant and sometimes the only possible ones in the treatment of patients with complicated widespread peritonitis, sepsis or compartment syndrome of urgent abdominal pathology and abdominal injuries.

The aim of our study was to conduct a systematic review and meta-analysis to compare the effectiveness of vacuum-assisted laparostomy with various variants of relaparotomy and laparostomy without negative pressure therapy in the treatment of patients with complicated by widespread peritonitis, sepsis or compartment syndrome of urgent abdominal pathology and abdominal injuries.

MATERIAL AND METHODS

Design and proper testing environment. A systematic literature search was conducted in accordance with the recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [30]. We carried out the analysis of non-randomized (since January 2007 until August 6, 2022) and randomized (without time limits for the start of the study and until August 6, 2022) studies from the electronic databases eLibrary, PubMed, Cochrane Library, Science Direct, Google Scholar Search, Mendeley in accordance with the recommendations of the Federal State Budgetary Institution Center for Healthcare Quality Assessment and Control of the Ministry of Health of the Russian Federation [31].

Criteria for inclusion and exclusion of original research in meta-analysis. Primary search strategy (without language restrictions): vacuum-assisted laparostomy, laparostomy, vacuum therapy, negative pressure therapy, "open abdomen", on-demand relaparotomy, programmed (planned) relaparotomy, intra-abdominal hypertension syndrome, abdominal compartment syndrome, – with subsequent exclusion from the query for experimental studies, literature reviews, clinical recommendations, case reports, non-cohort studies, incomplete articles/theses, manuscripts devoted to endoscopic (endoluminal) vacuum therapy, vacuum therapy of other localization, outside the abdominal cavity or comparing different varieties of vacuum-assisted laparostomy with each other. Additionally, we searched for articles from the references of the selected studies for sources not found during the initial search; the tables of contents of specialized journals were analyzed. Data extraction was performed independently by three researchers. Any disagreement regarding study selection was resolved by consensus.

The methodological quality of non-randomized studies was assessed on the Newcastle – Ottawa Scale (NOS) [31, 32]. The results of the systematic bias risk assessment were interpreted as follows:

- studies with 5 points or less (out of 9 possible) have a high risk of systematic errors [32];
- studies with 6 and 7 points – the average risk of systematic errors [32];
- studies with 8 and 9 points – low risk of systematic errors [32].

The methodological quality of randomized clinical trials was assessed according to the criteria of the current Guidelines of the Cochrane Community [33] and according to the methodology proposed by V.V. Omelyanovsky et al. (2019) [31].

Statistical data analysis was carried out using Microsoft Excel 2019 (Microsoft Corp., USA); PythonMeta software (China) was used to synthesize quantitative data.

The null hypothesis is based on the assumption that there are no differences in the treatment outcomes after vacuum-assisted laparostomy and various options of relaparotomy and laparostomy without negative pressure therapy among patients with urgent abdominal pathology and abdominal injuries complicated by widespread peritonitis, sepsis or compartment syndrome.

Statistical heterogeneity was assessed using the heterogeneity index I^2. If no significant heterogeneity was detected ($I^2 \leq 40$ %), then a fixed effect model (Mantel – Hansel method) was used to generalize the results [33]. Otherwise, a random effects model was used. The statistical significance of the findings was confirmed by the determination of a 95% confidence interval (95% CI) (if the level of statistical significance is $p < 0.05$, then the differences are statistically significant) [33].

To quantify the influence of various dichotomous parameters on the outcome of the event under study, the odds ratio (OR) was determined in retrospective studies, the relative risk (RR) was determined in randomized clinical trials (RCTs), prospective and combined stud-

ies [33]. Differences in treatment outcomes of different groups of patients were taken into account only for odds and relative risk ratio values other than 1. If the confidence interval for OR/RR included 1, then there was no statistically significant difference in the studied groups [33]. The values of OR/RR > 1 show that the reviewed surgical intervention among patients of the main group increased the probability of occurrence of the reviewed event compared with the control. If the calculated value of OR/RR is < 1, the reviewed intervention reduces the probability of occurrence of the reviewed event in comparison with the control [33].

In the meta-analysis of rates (the number of repeated surgical interventions), information about the rates of the phenomenon under study was summarized by determining rate ratios, which are determined by dividing the rate in the main intervention group by the rate in the control group [33].

The analysis of continuous data (the average duration of inpatient treatment, the average duration of surgical treatment, etc.) was performed using information about the mean values, their standard deviation in each of the comparison groups and the total number of patients in the corresponding comparison groups [33]. Summary statistics for continuous data were evaluated by the standard mean difference (SMD) using Hedges'g [33]. The value of SMD ≤ 0.40 corresponds to a small, SMD from 0.40 to 0.70 – moderate, SMD > 0.70 – a large effect value [33].

A qualitative assessment of the ***systematic publication error*** was carried out using a funnel plot. The asymmetry of the plot indicated a systematic publication error [33].

Study outcomes. Analysis in subgroups. Results

The stages of the evidence base search are presented in the PRISMA flowchart (Fig. 1). As a result, 33 studies were included in the systematic review (including 1 RCT, 5 prospective studies, 1 combined (pro- and retrospective) study, 5 prospective cohort studies, 2 combined cohort studies, 9 retrospective studies, 4 retrospective cohort studies, 1 case-series study, 5 descriptions of clinical cases). All cohort studies (12), including 1 RCT, are included in the meta-analysis.

SYSTEMATIC REVIEW

D. Perez et al. (2007) [34] prospectively analyzed the treatment outcomes of 37 patients with vacuum-assisted laparostomy performed with widespread perito-

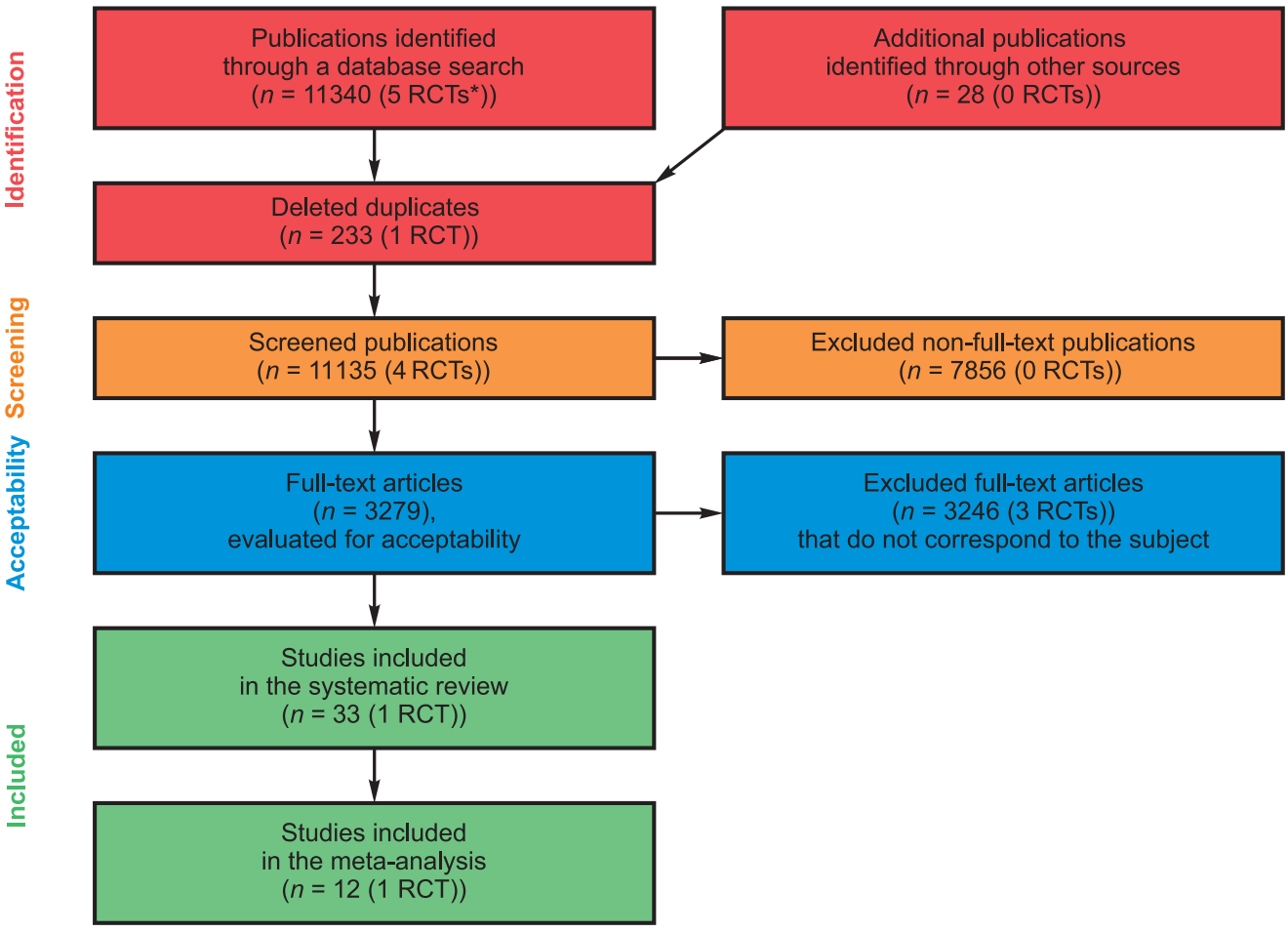

FIG. 1.
Stages of selection of the evidence base

nitis with severe abdominal sepsis (Mannheim peritonitis Index (MPI) > 29–21 (57 %) patient and/or IAHS – 16 (43 %) patients). These were critical patients (the SOFA (sequential organ failure assessment) score was 8.7 (7.2 to 11.5)); mortality was 65 % (24 patients: 14 died during the use of VAC-laparostomy, the rest – within 3 months after abdominal cavity closure). The average time spent in the intensive care unit (ICU) was 20.4 (12.3–35.2) days, while the duration of artificial lung ventilation (ALV) was 18.9 (12.6–29.9) days. The average duration of laparostomy was 22.7 (3–122) days with 3.8 intra-abdominal component replacements per patient (1–22 replacements). Complete closure of the fascia was achieved in 26 (70 %) patients [34].

M. Rao et al. (2007) [35] evaluated results of VAC-laparostomy in 29 patients; 10 (34.5 %) died; the average duration of vacuum therapy was 26 days (2–68 days range); the average stay in the ICU was 10.5 days (3–36 days range), 6 (20.7 %) patients developed intestinal fistulas with an average duration of 20 (2–50) days from the beginning of negative pressure therapy.

D.E. Barker et al. (2007) [36] retrospectively on 258 patients with vacuum-assisted laparostomy (116 patients with abdominal trauma, 120 patients with abdominal surgical pathology, 22 patients with vascular abdominal pathology) showed the following results. The length of hospital stay varied from 1 to 290 days (an average of 32 days); mortality was 26 % (67 patients); the average number of vacuum systems used per patient was 2.77 (planned relaparotomies were performed in 85 (59.9 %) patients with abdominal surgical and vascular pathology and in 27 (23.3 %) patients with abdominal trauma ($p < 0.05$; Pearson's χ^2)). Abdominal closure was successful among 226 (87.6 %) patients (without statistically significant differences in the three patient groups; primary muscular-aponeurotic closure was more frequently performed among patients who did not require intra-abdominal component replacement (89.4 % vs. 53.9 %; $p < 0.05$; Pearson's χ^2). The following complications were obtained in the groups of patients with abdominal trauma, abdominal surgical pathology and vascular abdominal pathology, respectively: enterocutaneous fistulas – 4 (3,4 %), 8 (6,7 %) and 1 (4.5 %), respectively; intra-abdominal abscesses – 5 (4,3 %), 4 (3,3 %) and 0 (0 %), respectively; intestinal obstruction – 1 (0,86 %), 1 (0,83 %) and 1 (4.5 %), respectively; IAHS – 1 (0,86 %), 1 (0,83 %) and 1 (4.5 %), respectively; evisceration – 1 (0,86 %), 0 (0 %) and 0 (0 %), respectively.

D. Wondberg et al. (2008) [37] conducted a prospective analysis of the treatment of 30 patients with abdominal sepsis who underwent vacuum-assisted laparostomy from 2004 to 2007. The mortality rate was 30 % (9/30); in 53 % of cases (16/30) it was impossible to perform muscular-aponeurotic closure, and 5 patients died before the conditions for laparostomy closure appeared; in 2 cases intestinal fistulas developed (6.66 %) [37]. The average number of intra-abdominal component replacements was 3 (1–10); the average duration of stay in the ICU was 7 (1–40), in hospital – 50 (18–101) [37]. The authors conclude that the use of this technique allows to achieve good results in this cohort of patients [37].

A.I. Amin et al. (2009) [17] with the help of 20 patients with VAC-laparostomy and the APACHE II Score of 16.7 ± 1.0 points (suspected hospital mortality – 37.2 ± 5.25 %) prospectively showed that primary closure was achieved in 15 (75 %) patients for 4.53 ± 1.64 days, mortality was 0 %, duration of stay in ICU – 19.8 ± 4.48 days, intestinal fistulas developed in 2 (10 %) patients.

J. Horwood et al. (2009) [38] studied 27 patients with VAC-laparostomy (the average ASA (American Society of Anesthesiologists) score – 3.75) and prospectively showed that the mortality rate was 37 % (10/27), and this is statistically significantly less than the suspected mortality as per the P-POSSUM scale, widely used in the UK; the average number of relaparotomies and intra-abdominal component replacements – 4, 2 (out of 17 survivors; 11.7 %) patients developed enterocutaneous fistulas, and for 5 (29.4 %) patients a delayed abdominal closure was performed. The authors state that vacuum-assisted laparostomy is associated with fewer complications and lower mortality among patients with IAHS and peritonitis [10, 38].

L. López-Quintero et al. (2010) [39] conducted a retrospective study of 19 patients with abdominal sepsis who underwent VAC-laparostomy: the average stay of patients in the ICU was 5.2 days (2–9 days range, ± 2), in hospital – 24.7 days (10–45 days range, ± 9.6), mortality was 26.3 % (5 patients), the average duration of vacuum therapy – 12.7 days (5–33 days range, ± 8.2), the average number of intra-abdominal component replacements – 3.9 (2–6 replacements range, ± 1.1). Among the survivors, the final closure of the abdominal cavity was achieved in 100 % of cases with half of them within 23 days; the incidence of enterocutaneous fistulas was 26.3 % (5 patients), acute intestinal obstruction (AIO) – 21 % (4 patients), postoperative hernias – 50 % (7 patients).

M. Schmelzle et al. (2010) [5] when retrospectively analyzing treatment results of 49 patients with secondary peritonitis (with an average value of MPI of 28 (10–44)) in Germany, who underwent "open abdomen" vacuum drainage for more than 7 days with negative pressure of 75–125 mm Hg, the following results were obtained: the average duration of stay in the ICU was 40 (0-197) days in hospital – 84 (14–197) days; 20 patients died (mortality – 40.8 %, and non-fecal peritonitis at the first relaparotomy was an independent prognostic factor for greater survival; $p = 0.031$), with VAC-laparostomy over 7 days, the possibility of delayed muscular-aponeurotic closure was 22.4 % (11 patients) [5]. The average number of relaparotomies was 4.9 (2–14), and 0.9 (0–5) after vacuum application [5]. The incidence of enterocutaneous fistula formation was 22.4 % (11 patients; 4 of them had multiple fistulas); performing relaparotomy after vacuuming statistically significantly increased the risk of this complication ($p < 0.001$) and reduced the possibility of successful muscular-aponeurotic closure of the abdominal cavity ($p = 0.033$) [5]. The authors emphasize that it is necessary to strive for earlier closure of the abdominal cavity, if possible, to re-

duce the likelihood of complications; at the same time, the risk of fistulas is associated not so much with the duration of vacuum therapy as with the number of repeated interventions [5].

R. Kafka-Ritsch et al. (2012) [40] conducted a retrospective study of 160 patients, most of whom (78 %) had signs of abdominal sepsis (median MPI – 25 (5–43)). The mortality rate was 20.6 % (33 patients). Factors increasing mortality were MPI > 25 (17 vs. 9 %; $p = 0.05$), extended volume of surgical intervention and male gender [40]. Delayed abdominal closure was achieved in 76 % of cases (121 patients); a single-factor analysis showed that the frequency of delayed closure was higher among women (86 vs. 69 %; $p = 0.04$) and among patients with limited primary surgery (e. g., resection of a section of the intestine without reconstruction) (93 vs. 62 %; $p = 0.00$) and lower among patients with relaparotomies (65 vs. 83 %; $p = 0.01$), when vacuum treatment lasted more than 5 days (67 vs. 81 %; $p = 0.04$), among patients with "open abdomen" according to M. Bjork classification type 3 or 4 (39 vs. 80 %; $p = 0.002$), among patients with pre-existing or formed fistulas during laparostomy (30 vs. 79 %; $p = 0.001$), as well as among patients with pancreatitis or pancreatic fistula (33 %; $p = 0.01$) [40]. Abscesses occurred among 13 (8 %) patients, 31 (19 %) – infections of the surgical site, enterocutaneous fistulas – 5 (3 %) patients [40].

L. Pérez Domínguez et al. (2012) [41] retrospectively analyzed the results of the use of VAC-laparostomy for 23 patients with secondary peritonitis; the number of abdominal set replacements averaged 3.1 (1–7 range), while the average duration of vacuum treatment was 14.8 (2–43) days before laparotomy wound closure; primary closure was achieved in 18 of 21 patients (85.7 %). The average duration of hospital stay was 110.1 (8–163) days, the mortality rate was 26 % (6 patients) [41]. Complications occurred in 7 (30.4 %) cases: in 3 (13 %) – intra-abdominal abscesses, in 4 (17.4 %) – enterocutaneous fistulas, in 1 (4.3 %) – evisceration [41]. The authors state that vacuum laparostomy is easy to use with an acceptable level of complications, and its wider use should be expected in the future [41].

V.N. Obolensky et al. (2013) [25] described a clinical case of successful treatment of a 35-year-old patient with duodenal ulcer bleeding, in whom, associated with the relapse and ineffectiveness of endohemostasis, the surgery revealed penetration of the ulcer into the head of the pancreas with bleeding from the gland vessels and with the formation of an inflammatory infiltrate involving the hepatic-duodenal ligament, common bile duct, and gallbladder. 2/3 gastric resection with gastroenteroanastomosis according to Hofmeister – Finsterer, cholecystectomy, lavage and drainage of the abdominal cavity were performed [25]. On the 5th day, due to negative dynamics, a relaparotomy was performed, pancreatic head necrosis and enzymatic peritonitis were revealed; duodenal stump and gastroenteroanastomosis were consistent; the abdominal cavity was lavaged and drained with skin suturing [25]. In 4 days, a programmed lavage relaparotomy was performed, and positive dynamics was noted; the abdominal cavity was lavaged and drained with skin suturing [25]. But in another 6 days, bile began to flow from the postoperative wound, and a relaparotomy was performed; ongoing peritonitis and bile leakage from the stump of the cystic duct were detected, where the Tachocomb sponge was fixed; a vacuum-assisted laparostomy was carried out, after which the patient's condition stabilized, and on the 4th day after the vacuum was placed, peritonitis was eliminated, and the wound was sutured completely [25].

H.T. Hougaard et al. (2014) [42] conducted a retrospective analysis of the use of the VAC Abdominal Dressing System with ABThera continuous dosed muscular-aponeurotic traction system in 115 patients, the average frequency of intra-abdominal component replacement was 4 (1–36), the average duration of vacuum use before laparostomy closure was 7 (1–75) days, delayed muscular-aponeurotic closure was achieved in 92 % of cases (106/115), mortality was 17 % (20/115), and the frequency of intestinal fistula was 3.5 % (4/115).

P.V. Polenok (2016) [22] presented an original technique of temporary abdominal cavity closure using a negative pressure system and applied it in 5 patients with secondary peritonitis and severity of condition as per the APACHE II Scale of 18–24 points; intra-abdominal pressure level in 4 out of 5 cases exceeded 15 mm wg at the end of the first laparotomy. The number of laparotomies among 3 patients was 4, among 2 patients – 5 [22]. By the time of laparostomy elimination, all patients had a satisfactory condition of the anterior abdominal wall tissues, no signs of suppuration and devitalization of tissues, which allowed to complete the last relaparotomy by layer-by-layer suturing of tissues without tension with complete reconstruction of the anterior abdominal wall [22].

D.S. Zemlyakov et al. (2016) [2] used NPWT system – Vivano Med-Abdominal Kit in 8 patients with widespread purulent peritonitis and noted a rapid decrease in intra-abdominal pressure, relief of peritonitis, and postoperative wound cleansing. The authors consider tertiary peritonitis with progressive abdominal sepsis as indications for this method [2].

O.V. Pervova et al. (2016) [43] presented a clinical case of successful 98-day treatment of a patient with total infected pancreatic necrosis, omental abscess, retroperitoneal phlegmon, widespread enzymatic peritonitis, abdominal sepsis, who underwent laparotomy, necrosequestrectomy, autopsy and drainage of retroperitoneal phlegmon, omentobursostomy, nasointestinal intubation, lavage, drainage of the abdominal cavity, laparostomy. The first programmed relaparotomy revealed progression of a purulent-destructive process in the pancreas, retroperitoneal tissue, necrosis of the transverse and ascending colon, transition of enzymatic peritonitis into fibrinous purulent one, which required performing right-sided hemicolectomy, ileostomy during the next planned relaparotomy [43]. Subsequently, due to vacuum flow-aspiration drainage of the omental bursa and retroperitoneum, the phenomena of abdominal sepsis and MSF were eliminated [43]. The authors note the important role of nega-

tive pressure technologies for adequate lavage of the infection focus [43].

I. Mintziras et al. (2016) [26] retrospectively, for 2005–2014, analyzed the results of using vacuum-assisted laparostomy in 43 patients with secondary peritonitis, the main causes of which were anastomotic dehiscence after resection (20 patients) or failure of sutured acute intestinal perforations (17 patients). The severity as per the APACHE II Score was 11 points, the average duration of VAC-laparostomy was 12 days (3–88 range). 20 (47 %) patients died from septic complications [26]. Enterocutaneous fistulas (ECFs) occurred among 16 (37.2 %) patients, and the authors found a direct relationship in their occurrence with the frequency of repeated interventions and the duration of vacuum therapy ($p < 0.001$) [26]. During the ROC analysis, it was found that the duration of VAC therapy over 13 days with negative pressure of 100 mmHg is a risk factor for the development of ECFs (81 % sensitivity, 74 % specificity) [26]. Besides, performing at least one relaparotomy after vacuuming statistically significantly increased the risk of this complication ($p < 0.001$), while gender, patient age, the cause of secondary peritonitis, the presence of cancer, the severity of peritonitis according to MPI had no statistically significant effect on fistula formation [26].

P. Sibaja et al. (2017) [21] retrospectively obtained the following results of 48 patients with abdominal sepsis and vacuum-instillation laparostomy: primary abdominal closure was achieved in 96 % of cases ($n = 46$) within an average of 6 days, no intestinal fistulas, a mortality was 8.33 % ($n = 4$); higher aponeurosis closure rates, lower mortality and reduced duration of hospital stay compared to the Bogota bag, the Wittmann patch and VAC-laparostomy without instillations were reported (cit. according to [6]).

K.A. Anisimova et al. (2018) [44] after a triplicate failure of the formed gastric tube after laparoscopic longitudinal resection of the abdomen and two sutures in a patient with overweight and metabolic syndrome (arterial hypertension, type 2 diabetes mellitus, dyslipidemia), the NPWT system was implanted, which helped to relief peritonitis and achieve wound cleansing for secondary suturing.

D.D. Sichinava et al. (2020) [45] presented a clinical case of treatment of a patient with widespread peritonitis complicated by an unformed biliodigestive fistula using a negative pressure system, which led to the formation of an external fistula, relief of widespread peritonitis and abdominal cavity closure.

V. Müller et al. (2020) [8] in their prospective study of 39 patients (2/3 with fecal peritonitis as a result of intestinal perforation or anastomosis failure), mortality was 10 % ($n = 4$); intra-abdominal bleeding occurred in 1 patient as a result of vacuum therapy; a primary muscular-aponeurotic closure was not achieved in 11 (28 %) patients. The authors note that patients with anastomosis failure required 2 or more relaparotomies (2–9) [8].

I.B. Uvarov et al. (2021) [46] presented a clinical case of anastomosis failure on the 6th day after laparoscopic low anterior rectal resection with total mesorectumectomy, ileostomy, colorectal anastomosis with a circumferential stapler. During relaparotomy, the abdominal cavity was lavaged without anastomosis separation with the installation of intra-abdominal and pelvic negative pressure therapy systems and transanal endoluminal vacuum drainage to the anastomosis site [46]. After two planned relaparotomies, the authors noted complete relief of peritonitis [46]. The experience of using combination therapy with negative pressure in the treatment of a patient with anastomosis failure complicated by secondary widespread purulent peritonitis has given encouraging results [46].

META-ANALYSIS

The results of the meta-analysis are presented in Table 1.

T.K. Bee et al. (2008) [47] conducted a randomized clinical trial comparing two types of laparostomies: vacuum-assisted ($n = 31$) and using a vicryl mesh (polyglactin 910) ($n = 20$) without vacuum (Table 1), most of these patients were with abdominal trauma, did not receive statistically significant differences in mortality and complications, and the percentage of successful abdominal closure.

S. Batacchi et al. (2009) [9] compared a vacuum variant of laparostomy ($n = 35$) with the Bogota bag in a prospective cohort study ($n = 31$). The primary diagnoses upon admission to the hospital were abdominal/vascular pathology (36.4 %), severe trauma (33.3 %) or abdominal sepsis (30.3 %) [9]. The authors obtained statistically significant differences in favor of vacuum therapy in terms of final abdominal closure, ALV duration, ICU stay and hospitalization in general, but did not receive statistically significant differences in hospital mortality [9]. After laparostomy in the interval from 8 to 24 hours, a statistically significantly faster decrease in intra-abdominal pressure ($p < 0.01$) and blood lactate ($p < 0.001$) was observed in the vacuum group [9]. A significant relative risk (RR) of mortality was observed at the age over 70 years (RR = 2.9), with intra-abdominal pressure values above 20 mmHg before decompression (RR = 3.4), the preoperative lactate level above 8 (RR = 2.8), and the postoperative lactate level above 6 (RR = 3.2); and vice versa, SAPS II and APACHE II Scales scores were not statistically significant in predicting mortality [9].

N.Y. Patel et al. (2011) [48] conducted a retrospective cohort study with 98 patients with abdominal/vascular pathology and abdominal trauma who were initially indicated for programmed re-intervention, who already had IAHS and could not fully close the anterior abdominal wall. At the same time, statistically significantly longer periods of abdominal cavity closure, ALV and hospital stay duration were obtained in the vacuum treatment group, but there is no data on the severity of patients in the study groups, early complications and mortality [48].

I. Pliakos et al. (2012) [49] conducted a retrospective cohort study of 58 patients, 27 of whom underwent VAC-laparostomy, the rest – other types of laparostomy without vacuum; at the same time, there was no statistical-

TABLE 1

THE MAIN CHARACTERISTICS OF THE PRIMARY STUDIES INCLUDED IN THE META-ANALYSIS

Author, publication year. Design and quality of research	Basic data and research results	Compared treatment methods in studies				
		1. VAC-laparostomy (main group)	Without vacuum therapy: 2. On-demand relaparotomy (1st control group)	Without vacuum therapy: 3. Planned relaparotomy (2nd control group)	Without vacuum therapy: 4. Other types of laparostomy (3rd control group)	OR, RR, 95% CI, p
1	2	3	4	5	6	7
Uvarov I.B. et al. (2022) [3] Prospective cohort study; 6 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	52	78	–	–	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	ASA 1 –10 (19.3 %)	ASA 1 – 31 (39.7 %)	–	–	**$p = 0.037$[a]** ASA 3_1 > ASA 3_2
		ASA 2 – 28 (53.8 %)	ASA 2 – 39 (50.0 %)			
		ASA 3 –14 (26.9 %)	ASA 3 – 8 (10.3 %)			
		MPI 1 (≤ 20 points) – 0 (0 %)	MPI 1 – 20 (25.6 %)			**$p < 0.001$[a]** MPI 3_1 > MPI 3_2
		MPI 2 (21–29 points) – 19 (36,5 %)	MPI 2 – 34 (43.6 %)			
		MPI 3 (≥ 30 points) – 33 (63.5 %)	MPI 3 – 24 (30.8 %)			
		APACHE II 1 (up to 10 points) – 13 (25.0 %)	APACHE II 1 – 30 (38.5 %)			$p = 0.236$[a] APACHE II_1 > APACHE II_2
		APACHE II 2 (11–15 points) – 33 (63.5 %)	APACHE II 2 – 37 (47.4 %)			
		APACHE II 3 (16–25 points) – 6 (11.5 %)	APACHE II 3 – 11 (14.1 %)			
	Complications as per the Clavien-Dindo classification, *n* (%)	2 – 52 (100 %)	2 – 78 (100 %)	–	–	$p = 1.000$[b]
		3a – 13 (25.0 %)	3a – 47 (60.3 %)			**$p < 0.01$[b]**
		3b – 0	3b – 19 (24.4 %)			**$p < 0.001$[b]**
		4a – 5 (9.62 %)	4a – 22 (28.2 %)			**$p < 0.001$[b]**
		4b (MSF) – 3 (5,8 %)	4b – 22 (28.2 %)			**$p < 0.001$[b]**
		d (the need to continue therapy after discharge from hospital) – 2 (3.9 %)	d – 37 (47.4 %)			**$p < 0.001$[b]**

TABLE 1 (*continued*)

1	2	3	4	5	6	7
	Switching from RLT/LS without VAC to VAC-LS, *n* (%)	–	11 (7 died (63.6 %))	–	–	–
	Number of reoperations on average per patient, *n*	3	1	–	–	–
	AC final closure period, days (M ± SD)	5.5	–	–	–	–
	Relieved sepsis by the end of surgical treatment, *n/N* (%)	9/11 (81.8 %)	5/24 (20.8 %)	–	–	–
	Average duration of treatment in ICU, days (M ± SD)	9.5 ± 1.5	10.2 ± 1.4	–	–	**$p = 0/011^{c}$**
	Average duration of inpatient treatment, days (M ± SD)	30.1 ± 10.3	32.7 ± 11.9	–	–	$p = 0.97^{c}$
	Mortality, *n* (%)	3 (5.8 %)	24 (30.8 %)	–	–	**$p < 0.001^{b}$**
Mutafchiyski V. M. et al. (2016) [20] Combined (retro- and prospective) cohort study; 5 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	49	–	–	59	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	$MPI_{av.}$ – 26.4 95% CI: 24.3–28.4 $APACHE\ II_{av.}$ – 19,3 95% CI: 17.9–20.6	–	–	"Closed" laparostomy with a mesh $MPI_{av.}$ – 27.3 95% CI: 25.1-29.4 $APACHE\ II_{av.}$ – 19,9 95% CI: 18.1-21.6	$p = 0.544^{d}$ MPI $p = 0.595^{d}$ APACHE II
	Complications, *n* (%)	Wound infections – 6 (12.2 %) ECF – 4 (8.1 %) Intra-abdominal abscesses – 5 (10.2 %) Necrotizing fasciitis – 1 (2 %)	–	–	Wound infections – 7 (11.8 %) ECF – 11 (18.6 %) Intra-abdominal abscesses – 19 (32.2 %) Necrotizing fasciitis – 9 (15.2 %)	$p = 0.952^{e}$ $p = 0.108^{e}$ $p = 0.142^{e}$ **$p = 0.012^{e}$**
	Number of reoperations on average per patient, *n*	3	–	–	3	$p = 0.409^{d}$
	AC final closure period, days (M ± SD)	8.8 ± 8.8	–	–	10 ± 13.7	$p = 0.209^{d}$
	Average duration of treatment in ICU, days (M ± SD)	6.1 ± 6.31	–	–	10.6 ± 8.43	**$p = 0.002^{d}$**
	Average duration of inpatient treatment, days (M ± SD)	15.1 ± 11.85	–	–	25.9 ± 20	**$p < 0.001^{r}$**
	Mortality, *n* (%)	14 (28.57 %)	–	–	31 (52.54 %)	**$p = 0.021^{e}$**

TABLE 1 (*continued*)

1	2	3	4	5	6	7
Bleszynski M.S. et al. (2016) [14] Retrospective cohort study; 6 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	136	75	–	–	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	APACHE IV – 86 ± 23	APACHE IV – 90 ± 25	–	–	$p > 0.05$
	Number of reoperations on average per patient, *n*	4	2	–	–	–
	Average duration of treatment in ICU, days (M ± SD)	15.3 ± 16	10.2 ± 11	–	–	**$p = 0{,}006$**[e]
	Average duration of inpatient treatment, days (M ± SD)	61.8 ± 57	40.8 ± 33	–	–	**$p = 0{,}008$**[e]
	Mortality, *n* (%)	31 (22.8 %)	29 (38.7 %)	–	–	OR = 0.41 95% CI: 0.21–0.81 **$p = 0.012$**[f]
Bee T.K. et al. (2008) [47] RCT, high overall risk of systematic errors (C); 3 points (out of 5) as per the Jadad Scale	Number of patients	31	–	–	20	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	SBP at admission – 112 ± 31.5 GCS – 13 ± 3.9 ISS – 30 ± 9.9	–	–	"Closed" laparostomy with a mesh SBP – 104 ± 36.3 GCS – 13.2 ± 3.6 ISS – 30 ± 9.9	–
	Complications, *n* (%)	ECF – 6 (19,35 %) Intra-abdominal abscesses – 12 (38.7 %) Evisceration – 2 (6.45 %)	–	–	ECF – 1 (5 %) External pancreatic fistula – 1 (5 %) Intra-abdominal abscesses – 9 (45 %)	$p_{ECF} = 0.14$ [b]
	Number of reoperations on average per patient, *n*	2	–	–	–	–
	Number of patients with AC final closure after LS, *n* (%)	9 (29 %)	–	–	5 (25 %)	$p = 0{,}14$[b]
	Mortality, *n* (%)	8 (26 %)	–	–	5 (25 %)	$p = 1{,}0$[b]

TABLE 1 (*continued*)

1	2	3	4	5	6	7
Batacchi S. et al. (2009) [9] Prospective cohort study; 6 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	35	–	–	31	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	APACHE II – 21.6 ± 6.5 SAPS II – 52.4 ± 17.8	–	–	"Closed" laparostomy with a Bogota bag APACHE II – 23.2 ± 7.1 SAPS II – 49.1 ± 17.5	p_{APACHE} = 0.298[d] p_{SAPS} = 0.274[d]
	AC final closure period, days (M ± SD)	4.4 ± 1.8	–	–	6.6 ± 3.7	p = **0.025**[d]
	Average duration of ALV, days (M ± SD)	7.1 ± 5.4	–	–	9.9 ± 6.5	p = **0.039**[d]
	Average duration of treatment in ICU, days (M ± SD)	13.3 ± 5.2	–	–	19.2 ± 9.6	p = **0.024**[d]
	Average duration of inpatient treatment, days (M ± SD)	28.5 ± 4.7	–	–	34.9 ± 8.8	p = **0.019**[d]
	Mortality, *n* (%)	8 (22.9 %)	–	–	11 (35.4 %)	**p** = 0.288[g]
Patel N.Y. et al. (2011) [48] Retrospective cohort study; 4 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	15	–	34	49	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	–	–	"Closed" laparostomy with skin suturing	"Closed" laparostomy with a Bogota bag	–
	Number of reoperations on average per patient, *n*	2 (1–17)	–	1 (1–4)	2 (1–9)	***p* = 0.003**[c]
	AC final closure period, days (Me (min-max))	5 (2–69)	–	2 (1–7)	4 (1–24)	***p* = 0.001**[c]
	Number of patients with AC final closure after LS, *n* (%)	12 (80 %)	–	33 (97 %)	45 (91.8 %)	–
	ALV average duration, days (Me (min-max))	14 (4–60)	–	4 (1–35)	11 (1–49)	***p* = 0.001**[c]
	Average duration of inpatient treatment, days (Me (min-max))	29 (5–109)	–	16 (5–85)	23 (5–81)	***p* = 0.012**[c]

TABLE 1 (*continued*)

1	2	3	4	5	6	7
Rodrigues Jr. A.C. et al. (2015) [50] Retrospective cohort study; 4 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	17	–	–	10	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	–	–	–	"Closed" laparostomy with a Bogota bag	–
	Number of reoperations on average per patient, *n*	2	–	–	2	***p*** = 0.3[b]
	AC final closure period, days (M ± SD)	7.52 ± 9.03	–	–	10.8 ± 14.46	***p*** = 0.23[e]
	Number of patients with AC final closure after LS, *n* (%)	16 (94.1 %)	–	–	8 (80 %)	***p*** = 0.98[b]
Cherdantsev D.V. et al. (2016) [13] Prospective cohort study; 6 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	26	–	–	30	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	Sepsis – 6 (23 %) Severe sepsis – 12 (46.2 %) Septic shock – 8 (30.8 %) MPI > 25	–	–	Sepsis – 14 (46.7 %) Severe sepsis – 10 (33.3 %) Septic shock – 6 (20 %) MPI > 25	–
	Number of reoperations on average per patient, *n*	4	–	–	6	–
	Mortality, *n* (%)	6 (23 %)	–	–	14 (46.7 %)	–

TABLE 1 (*continued*)

1	2	3	4	5	6	7
Coccolini *F.* et al. (2017) [29] Prospective cohort study; 6 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	1 – 163 2 – 47	–	–	3 – 117 4 – 42	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	1. Classical VAC-LS 2. Barker's vacuum-packing technique	–	–	3. "Closed" laparostomy with a Bogota bag + "zip" on the skin 4. Wittmann patch	–
	Complications, *n* (%)	Complications in general/ECF 1– 53/22 (32.5 %/13.5 %) 2 – 21/1 (43.9 %/2.4 %)	–	–	Complications in general/ECF 3 – 42/9 (35.8 %/7.4 %) 4 – 25/7 (58.8 %/17.6 %)	–
	Number of reoperations on average per patient, *n*	1 – 1 2 – 1	–	–	3 – 1 4 – 0	–
	AC final closure period, days (M ± SD)	1 – 5.0 ± 4.1 2 – 6.6 ± 7.2	–	–	3 – 5.0 ± 4.4 4 – 6.6 ± 4.8	–
	Number of patients with AC final closure after LS, *n* (%)	1 – 140 (85.7 %) 2 – 36 (75.6 %)	–	–	3 – 97 (83.2 %) 4 – 33 (79.4 %)	–
	Mortality, *n* (%)	1 – 23 (14.3 %) 2 – 11 (24.4 %)	–	–	3 – 20 (16.8 %) 4 – 9 (20.6 %)	–

TABLE 1 (*continued*)

1	2	3	4	5	6	7
Pliakos I. et al. (2012) [49] Retrospective cohort study; 5 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	27	–	–	31	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	There is no statistically significant difference in the severity of condition as per the APACHE II Scale, age, and gender of patients. APACHE II $_{av.}$ – 18.6	–	–	APACHE II $_{av.}$ – 17.3 Bogota bag – 8 "Zip" – 8 Wittmann patch – 6 Non-absorbable mesh – 5 Others – 4	–
	Complications, *n* (%)	No	–	–	ECF – 17 (54.8 %)	***p* < 0.001**
	Number of reoperations on average per patient, *n*	4	–	–	16	***p* < 0.001**
	AC final closure period, days (M ± SD)	20	–	–	14	***p* < 0.001**
	Number of patients with AC final closure after LS, *n* (%)	22 (81.5 %)	–	–	9 (29 %)	***p* < 0.001**
	Mortality, *n* (%)	10 (37 %)	–	–	14 (45 %)	$p > 0.05$

TABLE 1 (*continued*)

1	2	3	4	5	6	7
Anisimov A.Yu. et al. (2017) [27] Prospective cohort study; 6 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	8	–	14	–	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	APACHE II – 17–27 Pancreatogenic sepsis – 100 %	–	APACHE II – 14–24 Pancreatogenic sepsis – 92.86 %	–	–
	Complications, *n* (%)	Bleeding – 1 (12.5 %) ECF – 1 (12.5 %)	–	–	–	–
	Number of reoperations on average per patient, *n*	5	–	3	–	**$p = 0.010448$**
	Average duration of inpatient treatment, days (M ± SD)	43.3 ± 13.6	–	37.0 ± 15.8	–	$p = 0.347353$
	Mortality, *n* (%)	4 (50 %)	–	9 (64.3 %)	–	–
Pogorelov M.V. et al. (2020) [23] Combined cohort study; 5 points as per the Newcastle – Ottawa Quality Assessment Scale	Number of patients	4	–	12	–	–
	Brief description of patients (MPI, severity of the condition according to ASA, APACHE Scales, etc.)	With toxic peritonitis phase – 3	–	Less severe patients	–	–
	Complications, *n* (%)	No	–	Adhesive AIO – 3 (25 %) ECF – 1 (8.3 %) Intra-abdominal abscesses – 2 (16.7 %)	–	–
	Number of reoperations on average per patient, *n*	2	–	2	–	–
	Mortality, *n* (%)	1 (25 %)	–	0 (0 %)	–	–

Note. [a] – Friedman test (χ^2) for multiple arbitrary-sized contingency tables; [b] – Pearson's chi-square (X^2) test; [c] – Kruskal – Wallis H test (for comparing more than two groups; [d] – Mann – Whitney U test; [e] – Student's t-test; [f] – F-test (χ^2); [g] – Fisher's exact test; 95% CI – 95% confidence interval; RLT – relaparotomy; LS – laparostomy; AC – abdominal cavity; ECF – enterocutaneous fistula; GCS – Glasgow Coma Scale; ISS – Injury Severity Score.

ly significant difference in the severity of the condition as per the APACHE II Scale, age, gender of patients. The authors obtained statistically significant differences in the average duration of laparostomy (20 days with vacuum vs. 14 days with other types; $p < 0.001$), the number of reoperations (4 vs. 16, respectively; $p < 0.001$), the number of patients with successful primary laparostomy closure (22 vs. 9; $p < 0.001$), the frequency of enterocutaneous fistula formation (0 vs. 17, respectively; $p < 0.001$), but no statistically significant differences in mortality were obtained (10 vs. 14, respectively) [49].

A.C. Rodrigues Jr. et al. (2015) [50] compared vacuum laparostomy ($n = 17$) with the Bogota bag ($n = 10$) among survived patients with abdominal sepsis in a retrospective cohort study and obtained no statistically significant diffe rence in the time and number of successful closure of the abdominal cavity, as well as in the number of reoperations.

D.V. Cherdantsev et al. (2016) [13] compared traditional and vacuum laparostomy in a prospective cohort study and concluded that the use of the latter makes it possible to improve the intra-abdominal situation in a shorter time, reducing the number of reoperations, which helps to minimize the number of systemic and local complications and improve treatment outcomes of patients with severe abdominal sepsis [13].

V.M. Mutafchiyski et al. (2016) [20] in their combined (retro- and prospective) cohort study compared the results of treatment of patients with widespread peritonitis who underwent vacuum-assisted laparostomy in the main group (prospective study) and closed laparostomy using a permeable synthetic mesh in the control group. VAC-laparostomy showed statistically significantly lower rates of necrotizing fasciitis (2 % vs. 15.2 %, respectively; $p = 0.012$), overall mortality (28.57 % vs. 52.54 %, respectively; $p = 0.021$), shorter ICU stays (6.1 vs. 10.6 days, respectively; $p = 0.002$) and hospitalization (15.1 vs 25.9 days; $p = 0.000$). If a VAC-laparostomy should be prolonged for more than 9 days, its combination with a system of continuous dosed muscular-aponeurotic traction is recommended [20].

M.S. Bleszynski et al. (2016) [14] carried out a retrospective comparative analysis of the results of VAC-laparostomy and on-demand relaparotomy among patients having abdominal sepsis, the main cause (59 %) of which were intestinal perforations, mesenteric ischemia and anastomosis failure. The authors have obtained encouraging results concerning reducing mortality in VAC-laparostomy among septic patients, and they emphasize the difficulties in timely indications for on-demand relaparotomy [14].

A.Yu. Anisimov et al. (2017) [27] used the NPWT technique (Vivano Tec, Germany) for 8 patients with pancreatogenic sepsis, and in a control group of 14 subjects they used traditional omentobursostomy; otherwise, the volume of surgical interventions and conservative measures did not differ; the groups were comparable in the severity of patients as per the APACHE II, Balthazar Scales, clinical signs of sepsis as per R.C. Bone classification. Later there were 3 to 5 planned relaparotomies; on-demand reoperations were performed only when bleeding occurred [27]. Negative pressure ensured more effective continuous evacuation of exudate, early relief of systemic inflammatory reaction syndrome and IAHS [27]. Mortality in the main group was 50 % (4 of 8) versus 64.3 % (9 of 14) in the comparison group [27]. According to the authors, the NPWT method has its shortcomings and complications: petechial and arrosive bleeding occurred in 1 (12.5 %) patient, and in 1 (12.5 %) case the postoperative period was complicated by the development of intestinal fistula, but no statistically significant differences in the incidence of the above complications and mortality rate with and without NPWT were obtained by the authors [27].

In 2017, the first results of an international study (supervised by F. Coccolini) devoted to the epidemiology, indications and effectiveness of laparostomy in the global surgical community were published in the International Register of Open Abdomen (IROA) [19, 29]. 402 patients were registered, 369 of them – adults. The average age of adult patients – 57.39 ± 18.37 years [19, 29]. The reasons for performing laparostomy were: peritonitis (in case of septic shock and inability to perform radical surgery due to the severity of the patient's condition; if a delayed anastomosis was necessary; in case of oedema of intestinal loops with suspected development of IAHS) – 48.7 % of cases; trauma – 20.5 %; vascular pathology/bleeding (rupture of the abdominal aortic aneurysm) – 9.4 %; ischemia (planned intestine revision in case of mesenteric ischemia and resection) – 9.1 %; severe pancreonecrosis with MSF and IAHS – 4.2 %; postoperative abdominal syndrome – 3.9 %; others – 4.2 % [19, 29]. The most common method of temporary closure was the use of commercial negative pressure systems – 44.2 %. Complications developed in 38 % of the patients, 10.5 % of which were intestinal fistulas. Primary muscular-aponeurotic closure was achieved in 82.8 % of patients. The mortality rate was 17.2 % [19, 29]. The duration of laparostomy – 5.39 ± 4.83 days. A direct linear correlation was found between the duration of laparostomy and complications (Pearson correlation coefficient = 0.326; $p < 0.0001$) with the development of enterocutaneous fistulas (Pearson correlation coefficient = 0.146; $p = 0.016$) [19, 29].

M.V. Pogorelov et al. (2020) [23] compared the results of treatment of 16 children with widespread purulent peritonitis, in 4 of whom they used the technique of local negative pressure and programmed lavages, 12 – only programmed relaparotomy. The authors concluded that laparostomy with intra-abdominal VAC-therapy is a reliable and effective method of treating widespread peritonitis with multiple interstitial abscesses, including tertiary peritonitis; it reduces intra-abdominal pressure more rapidly and causes fewer complications [23].

I.B. Uvarov et al. (2022) [3] conducted a prospective comparative non-randomized clinical study to evaluate the effectiveness of vacuum-assisted laparostomy with staged lavages in comparison with on-demand relapa-

rotomies among patients with secondary widespread postoperative peritonitis (Table 1). No adverse events of severe and moderate degree associated with the use of negative pressure therapy (bleeding, bedsores of hollow organ walls and intestinal fistula formation, intestinal obstruction) have been reported [3]. The use of VAC-laparostomy in comparison with on-demand relaparotomy provides more effective relief of abdominal sepsis, is associated with lower mortality, fewer complications and severity, shorter stay in the intensive care unit and hospital after closure of the abdominal cavity [3].

1. Comparison of vacuum-assisted laparostomy with other options for multistage surgical treatment by the number of necessary repeated operations.

A. Comparison with on-demand relaparotomy: 2 studies [3, 14] (Fig. 2).

The relative risk was 1.91 (95% CI: 0.54–6.77; I^2 = 0 %; fixed effects model).

There are no statistically significant differences between vacuum-assisted laparostomy and on-demand relaparotomy in the number of necessary repeated operations (p = 0.319).

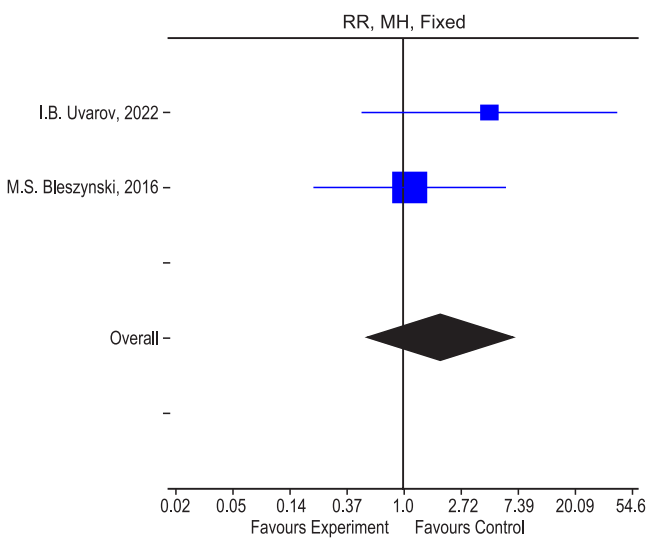

FIG. 2.

Forest plot showing the results of comparing vacuum-assisted laparostomy and relaparotomy "on demand" by the number of necessary repeated operations

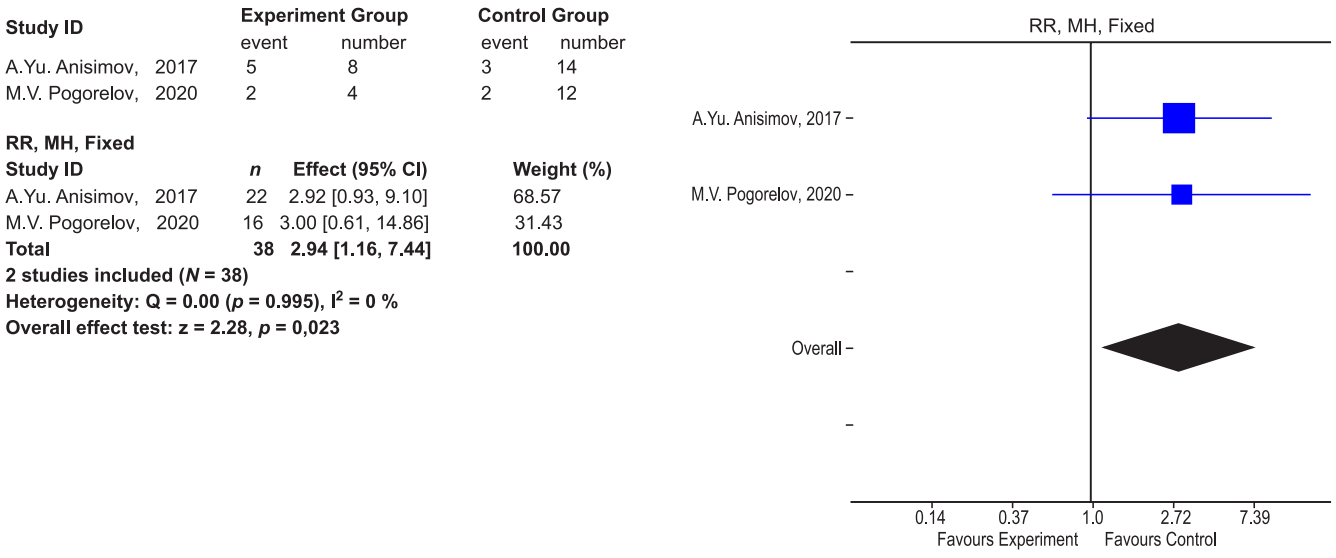

FIG. 3.

Forest plot showing the results of comparing vacuum-assisted laparostomy and programmed relaparotomy by the number of necessary repeated operations

B. Comparison with programmed relaparotomy.

A comparison is possible based on two studies [23, 27] (Fig. 3, 4).

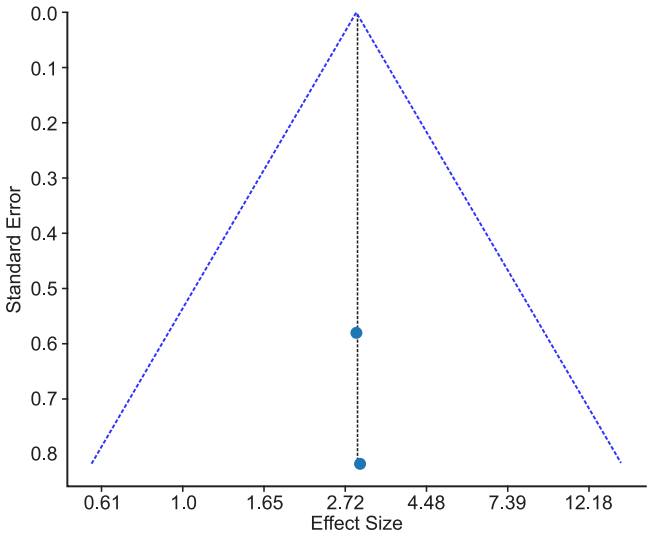

FIG. 4.

Funnel plot of evaluation of the systematic error of publications by the number of necessary repeated operations in vacuum-assisted laparostomy and programmed relaparotomy

In the study of N.Y. Patel et al. [48], information concerning the number of necessary repeated operations is presented in the form of median, minimum and maximum; comparison with the average number is impossible.

The relative risk was 2.94 (95% CI: 1.16–7.44; I^2 = 0 %; fixed effects model).

In the studies under consideration, the number of necessary reoperations during programmed relaparotomies is statistically significantly lower than during vacuum-assisted laparostomy (p = 0.023).

A symmetrical funnel plot indicates the absence of obvious systematic publication errors (no publication bias).

C. Comparison with other variants of laparostomy without vacuum: 5 studies [13, 20, 29, 49, 50] – 6 comparison groups (Fig. 5).

The relative risk was 0.57 (95% CI: 0.32–1.01; I^2 = 0 %; fixed effects model).

There are no statistically significant differences in the number of necessary reoperations with vacuum laparostomy and laparostomy without vacuum (p = 0.053).

2. Comparison of vacuum-assisted laparostomy with other variants of laparostomy without vacuum by the number of patients with successful closure of the abdominal cavity (Fig. 6).

The relative risk was 1.11 (95% CI: 0.90–1.36; I^2 = 67.37 %; random effects model).

There are no statistically significant differences in the number of patients with successful closure of the abdominal cavity after vacuum-assisted laparostomy or other variants of laparostomy without vacuum (p = 0.333).

3. Comparison of vacuum-assisted laparostomy with other multistage surgical treatment options by the average time of final closure of the abdominal cavity.

A. Comparison with on-demand relaparotomy: 2 studies [3, 14].

Study ID	Experiment Group		Control Group	
	event	number	event	number
V.M. Mutafchiyski, 2016	3	49	3	59
A.C. Rodrigues J., 2015	2	17	2	10
D.V. Cherdantsev, 2016	4	26	6	30
F. Coccolini, 2017	1	163	1	117
F. Coccolini, 2017	1	47	0	42
I. Pliakos, 2012	4	27	16	31

RR, MH, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	108	1.20 [0.25, 5.70]	9.93
A.C. Rodrigues J., 2015	27	0.59 [0.10, 3.55]	9.19
D.V. Cherdantsev, 2016	56	0.77 [0.24, 2.43]	20.33
F. Coccolini, 2017	280	0.72 [0.05, 11.36]	4.25
F. Coccolini, 2017	89	2.69 [0.11, 64.25]	1.93
I. Pliakos, 2012	58	0.29 [0.11, 0.75]	54.37
Total	**618**	**0.57 [0.32, 1.01]**	**100.00**

6 studies included (N = 618)

Heterogeneity: Q = 4.03 (p = 0.546), I^2 = 0 %

Overall effect test: z = 1.94, p = 0,053

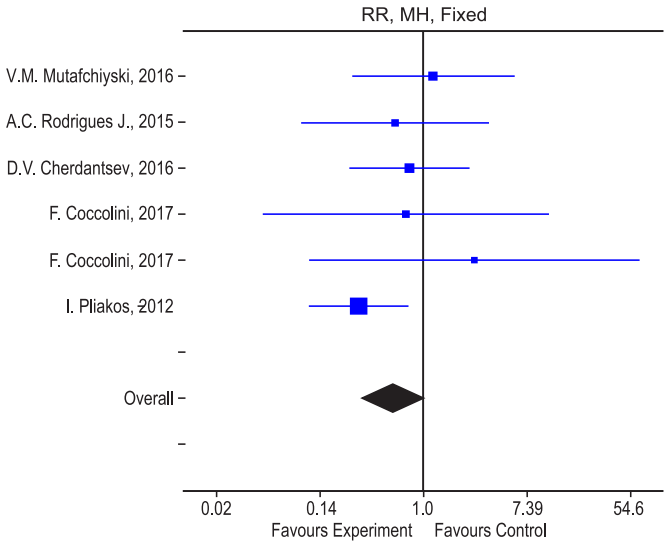

FIG. 5.

Forest plot showing the results of comparing vacuum-assisted laparostomy and laparostomy without vacuum by the number of repeated operations required

Study ID	Experiment Group		Control Group	
	event	number	event	number
T.K. Bee, 2008	9	31	5	20
N.Y. Patel, 2011	12	15	45	49
A.C. Rodrigues J., 2015	16	17	8	10
F. Coccolini, 2017	140	163	97	117
F. Coccolini, 2017	36	47	33	42
I. Pliakos, 2012	22	27	9	31

RR, MH, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
T.K. Bee, 2008	51	1.16 [0.45, 2.97]	4.13
N.Y. Patel, 2011	64	0.87 [0.67, 1.14]	1.98
A.C. Rodrigues J., 2015	27	1.18 [0.84, 1.64]	16.84
F. Coccolini, 2017	280	1.04 [0.93, 1.15]	27.99
F. Coccolini, 2017	89	0.97 [0.78, 1.22]	22.20
I. Pliakos, 2012	58	2.81 [1.57, 5.01]	8.86
Total	**569**	**1.11 [0.90, 1.36]**	**100.00**

6 studies included (*N* = 569)
Heterogeneity: Tau² = 0.036, Q = 15.32 (*p* = 0.009), I² = 67.37 %
Overall effect test: z = 0.97, *p* = 0,333

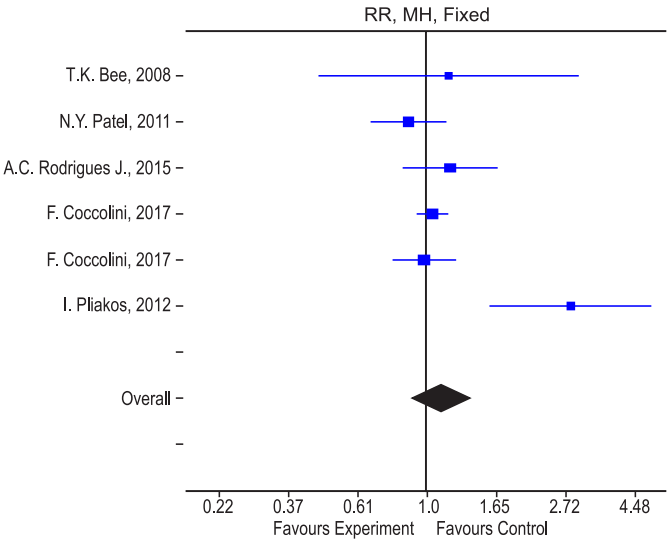

FIG. 6.
Forest plot showing the results of comparing vacuum-assisted laparostomy and laparostomy without vacuum by the number of patients with successful closure of the abdominal cavity

Study ID	Experiment Group			Control Group		
	mean	sd	number	mean	sd	number
V.M. Mutafchiyski, 2016	8.80	8.80	49	10.00	13.70	59
S. Batacchi, 2009	4.40	1.80	35	660	3.70	31
A.C. Rodrigues J., 2015	7.52	9.03	17	10.80	14.46	10
F. Coccolini, 2017	5.00	4.10	163	5.00	4.40	117
F. Coccolini, 2017	6.60	7.20	47	6.60	4.80	42

SMD, IV-Heg, Random

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	51	1.16 [0.45, 2.97]	4.13
S. Batacchi, 2009	64	0.87 [0.67, 1.14]	1.98
A.C. Rodrigues J., 2015	27	1.18 [0.84, 1.64]	16.84
F. Coccolini, 2017	280	1.04 [0.93, 1.15]	27.99
F. Coccolini, 2017	89	0.97 [0.78, 1.22]	22.20
Total	**570**	**–0.17 [–0.43, 0.09]**	**100.00**

5 studies included (*N* = 570)
Heterogeneity: Tau² = 0.040, Q = 7.76 (*p* = 0.101), I² = 48.48 %
Overall effect test: z = 1.31, *p* = 0,192

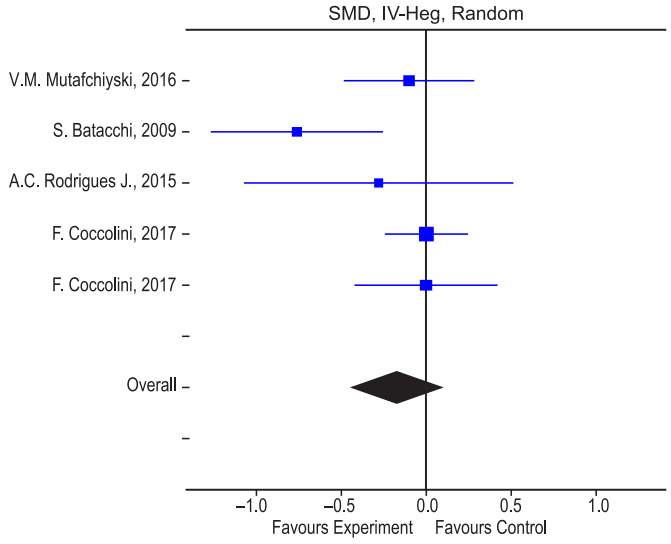

FIG. 7.
Forest plot showing the results of comparing vacuum-assisted laparostomy and other variants of laparostomy without vacuum according to the average timing of the final closure of the abdominal cavity

Comparisons cannot be done because one study provides data for one cohort [3], and the other does not [14].

B. Comparison with programmed relaparotomy: 3 studies [23, 27, 48].

It is impossible to make comparisons among the selected studies, because this information is not available in two studies [23, 27].

C. Comparison with other variants of laparostomy without vacuum: 4 studies [9, 20, 29, 50] – 5 comparison groups (Fig. 7).

SMD is –0.17 (95% CI: –0.43–0.09; I^2 = 48.48 %).

In the course of generalizing calculations of the above studies (I^2 statistics – 48.48 %; random effects model), it was revealed that **the average timing of the final closure of the abdominal cavity after vacuum-assisted laparostomy and other variants of laparostomy without vacuum did not differ** statistically significantly (p = 0.192).

4. Comparison of vacuum-assisted laparostomy with other multistage surgical treatment options for postoperative complications.

Study ID	Experiment Group		Control Group	
	event	number	event	number
V.M. Mutafchiyski, 2016	4	49	11	59
T.K. Bee, 2008	6	31	1	20
F. Coccolini, 2017	22	163	9	117
F. Coccolini, 2017	1	47	7	42
I. Pliakos, 2012	0	27	17	31

RR, MH, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	108	0.44 [0.15, 1.29]	24.53
T.K. Bee, 2008	51	3.87 [0.50, 29.80]	17.74
F. Coccolini, 2017	280	1.75 [0.84, 3.67]	26.63
F. Coccolini, 2017	89	0.13 [0.02, 1.00]	17.66
I. Pliakos, 2012	58	0.03 [0.00, 0.52]	13.44
Total	**586**	**0.53 [0.13, 2.12]**	**100.00**

5 studies included (*N* = 586)
Heterogeneity: Tau^2 = 1.740, Q = 17.48 (*p* = 0.002), I^2 = 77.12 %
Overall effect test: z = 0.90, *p* = 0,371

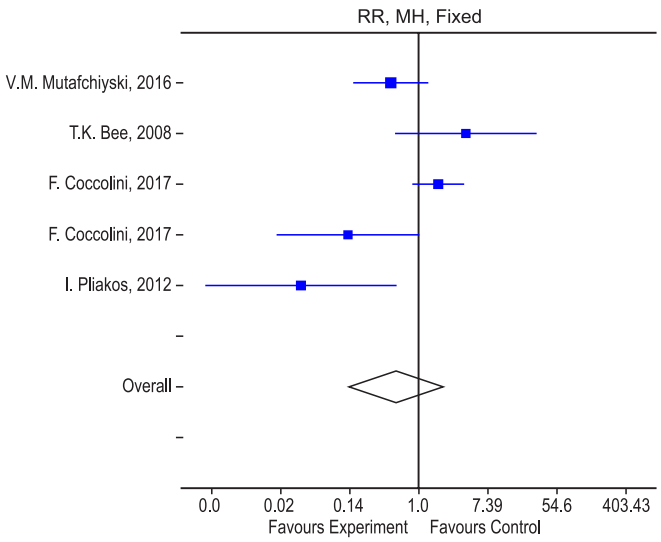

FIG. 8.
Forest plot showing the results of comparing vacuum-assisted laparostomy and laparostomy without vacuum by the frequency of development of enterocutaneous fistulas

Study ID	Experiment Group		Control Group	
	event	number	event	number
V.M. Mutafchiyski, 2016	5	49	19	59
T.K. Bee, 2008	12	31	9	20

RR, MH, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	108	0.32 [0.13, 0.79]	45.28
T.K. Bee, 2008	51	0.86 [0.45, 1.66]	54.72
Total	**159**	**0.55 [0.20, 1.52]**	**100.00**

2 studies included (*N* = 159)
Heterogeneity: Tau^2 = 0.382, Q = 3.34 (*p* = 0.068), I^2 = 70.02 %
Overall effect test: z = 1.16, *p* = 0,250

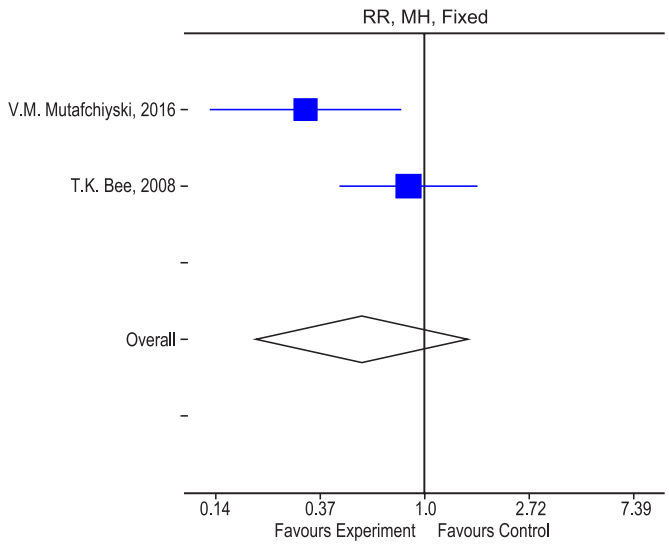

FIG. 9.
Forest plot showing the results of comparing vacuum-assisted laparostomy and laparostomy without vacuum by the frequency of intra-abdominal abscesses

A. Comparison with on-demand relaparotomy: 2 studies [3, 14].

In the presented studies, it is impossible to compare postoperative complications, because one study does not characterize them, and the second study does not provide any data.

B. Comparison with programmed relaparotomy: 3 studies.

In the presented studies [23, 27, 48], a comparison of postoperative complications is impossible, because there are no data on the compared cohorts.

C. Comparison with other variants of laparostomy without vacuum: 4 studies [20, 29, 47, 49] – 5 comparison groups.

In the presented studies, it is possible to compare enterocutaneous fistulas (Fig. 8):

The relative risk was 0.53 (95% CI: 0.13–2.12; I^2 = 77.12 %; random effects model).

There are no statistically significant **differences between vacuum-assisted laparostomy and other variants of laparostomy without vacuum by the frequency of development of enterocutaneous fistulas** (*p* = 0.371).

Study ID	Experiment Group			Control Group		
	mean	sd	number	mean	sd	number
I.B. Uvarov, 2022	9.50	1.50	52	10.00	1.40	78
M.S. Bleszynski, 2016	15.30	16.00	136	10.20	11.00	75

SMD, IV-Heg, Random

Study ID	*n*	Effect (95% CI)	Weight (%)
I.B. Uvarov, 2022	130	–0.48 [–0.84, –0.13]	49.14
M.S. Bleszynski, 2016	211	0.35 [0.07, 0.64]	50.86
Total	**341**	**–0.06 [–0.88, 0.76]**	**100.00**

2 studies included (*N* = 341)
Heterogeneity: Tau^2 = 0.322, Q = 12.93 (*p* = 0.001), I^2 = 92.26 %
Overall effect test: z = 0.14, *p* = 0,889

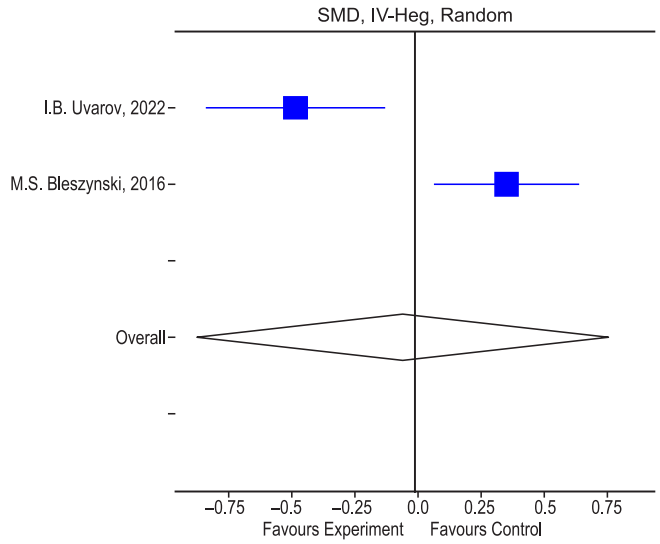

FIG. 10.
Forest plot showing the results of comparing vacuum-assisted laparostomy and relaparotomy "on demand" by the average length of treatment in the ICU

Study ID	Experiment Group			Control Group		
	mean	sd	number	mean	sd	number
V.M. Mutafchiyski, 2016	6.10	6.31	49	10.60	8.43	59
S. Batacchi, 2009	13.30	5.20	35	19.20	9.60	31

SMD, IV-Heg, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	108	–0.59 [–0.98, –0.20]	62.69
S. Batacchi, 2009	66	–0.77 [–1.27, –0.27]	37.31
Total	**174**	**–0.66 [–0.96, –0.35]**	**100.00**

2 studies included (*N* = 174)
Heterogeneity: Q = 0.30 (*p* = 0.588), I^2 = 0 %
Overall effect test: z = 4.21, *p* = 0,000

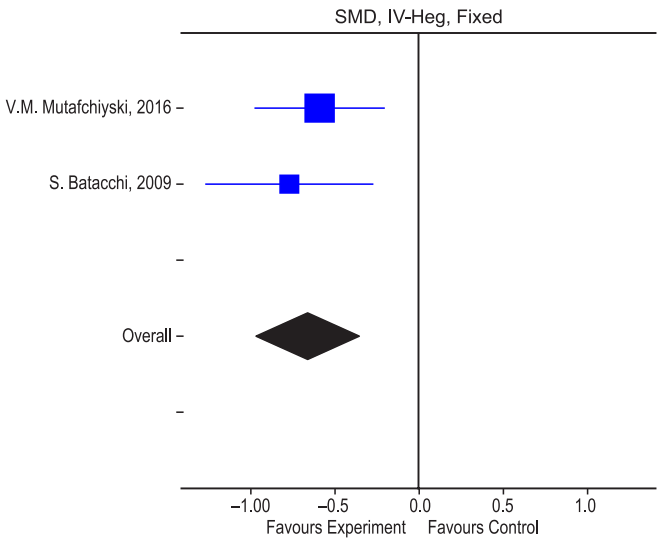

FIG. 11.
Forest plot showing the results of comparing vacuum-assisted laparostomy and other variants of laparostomy without vacuum by the average length of treatment in the ICU

D. Comparison by the frequency of intra-abdominal abscesses: 2 studies [20, 47] (Fig. 9).

The relative risk was 0.55 (95% CI: 0.20–1.52; I^2 = 70.02 %; random effects model).

There are no statistically significant **differences between vacuum-assisted laparostomy and other variants of laparostomy without vacuum by the frequency of intra-abdominal abscesses** (*p* = 0.250).

5. Comparison of vacuum-assisted laparostomy with other options for multistage surgical treatment according to the average duration of patients' stay in ICU.

A. Comparison with on-demand relaparotomy: 2 studies [3, 14] (Fig. 10).

SMD is –0.06 (95% CI: –0.88–0.76; I^2 = 92.26 %).

In the course of generalizing calculations of the above studies (I^2 statistics – 92.26 %; random effects model), it was revealed that the **average duration of stay in the ICU after vacuum-assisted laparostomy and on-demand relaparotomy did not differ** statistically significantly (*p* = 0.889).

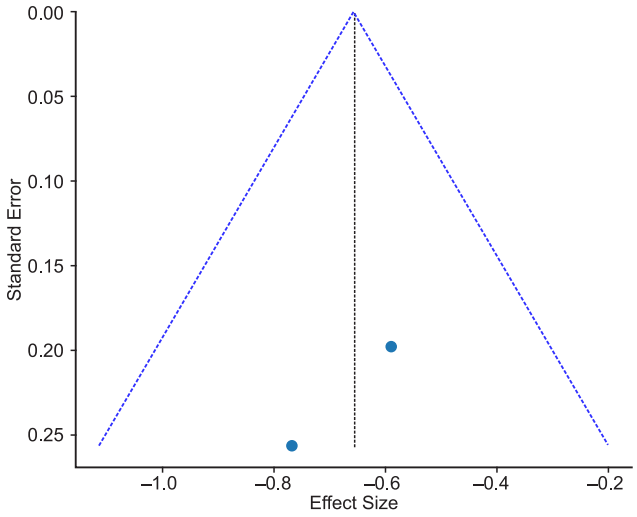

FIG. 12.

Funnel plot of evaluation of the systematic error of publications by the average duration of treatment in the ICU after vacuum-assisted laparostomy and other variants of laparostomy without vacuum

B. Comparison with programmed relaparotomy: 3 studies [23, 27, 48].

Comparison is not possible among the selected studies, because this information is not available.

C. Comparison with other variants of laparostomy without vacuum: 2 studies [9, 20] (Fig. 11, 12).

SMD is –0.66 (95% CI: –0.96÷–0.35; I^2 = 0 %).

In the course of generalizing calculations of the above studies (I^2 statistics – 0 %; fixed effects model), it was revealed that **the average duration of treatment in the ICU after vacuum-assisted laparostomy** was statistically significantly **less than with in other laparostomy variants without vacuum** (p = 0.000).

A symmetrical funnel plot indicates the absence of obvious systematic publication errors (no publication bias).

6. Comparison of vacuum-assisted laparostomy with other options for multistage surgical treatment according to the average duration of hospital stay.

A. Comparison with on-demand relaparotomy: 2 studies [3, 14] (Fig. 13).

The SMD is 0.10 (95% CI: –0.53–0.74; I^2 = 87.32 %).

In the course of generalizing calculations of the above studies (I^2 statistics – 87.32 %; random effects model), it was revealed that the **average duration of inpatient treatment after vacuum-assisted laparostomy and on-demand relaparotomy did not differ** statistically significantly (p = 0.749).

B. Comparison with programmed relaparotomy: 3 studies.

Comparison among the selected studies is impossible, because only one of them provides the average duration of inpatient treatment and the standard deviation [27], the other shows the median with the maximum and minimum duration of treatment [48], and the third has no information at all [23].

B. Comparison with other variants of laparostomy without vacuum: 2 studies [9, 20].

The study [48] shows the median with the maximum and minimum duration of treatment (Fig. 14, 15).

The SMD is –0.74 (95% CI: –1.05÷–0.43; I^2 = 0 %).

In the course of generalizing calculations of the above studies (I^2 statistics – 0 %; fixed effects model), it was revealed that **the average duration of inpatient treatment after vacuum-assisted laparostomy was statistically sig-**

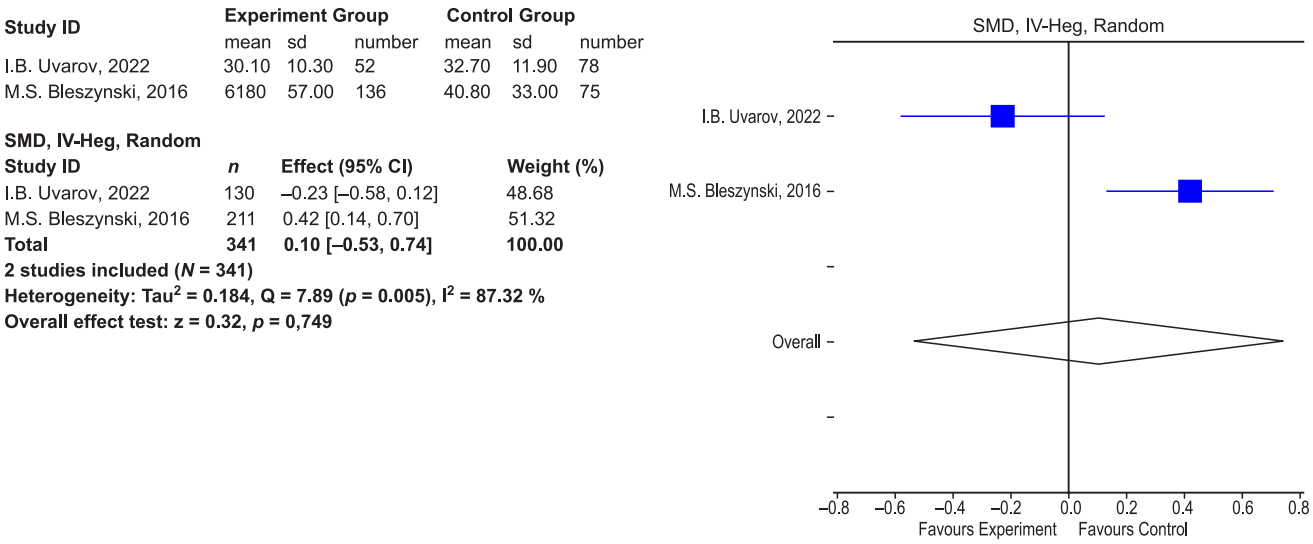

FIG. 13.

Forest plot showing the results of comparing vacuum-assisted laparostomy and relaparotomy "on demand" by the average duration of inpatient treatment

Study ID	Experiment Group			Control Group		
	mean	sd	number	mean	sd	number
V.M. Mutafchiyski, 2016	15.10	11.85	49	25.90	20.00	59
S. Batacchi, 2009	28.50	4.70	35	34.90	8.80	31

SMD, IV-Heg, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	108	–0.64 [–1.03, –0.25]	63.21
S. Batacchi, 2009	66	–0.91 [–1.42, –0.40]	36.79
Total	**174**	**–0.74 [–1.05, –0.43]**	**100.00**

2 studies included (*N* = 174)
Heterogeneity: Q = 0.71 (*p* = 0.401), I^2 = 0 %
Overall effect test: z = 4.69, *p* = 0,000

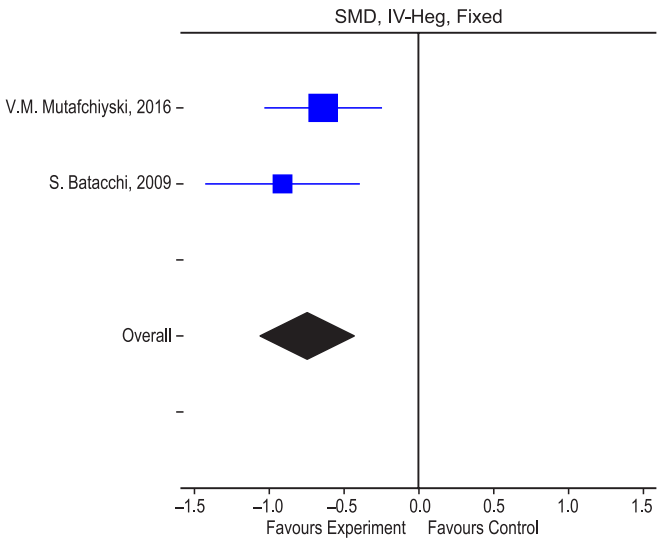

FIG. 14.
Forest plot showing the results of comparing vacuum-assisted laparostomy and other variants of laparostomy without vacuum by the average duration of inpatient treatment

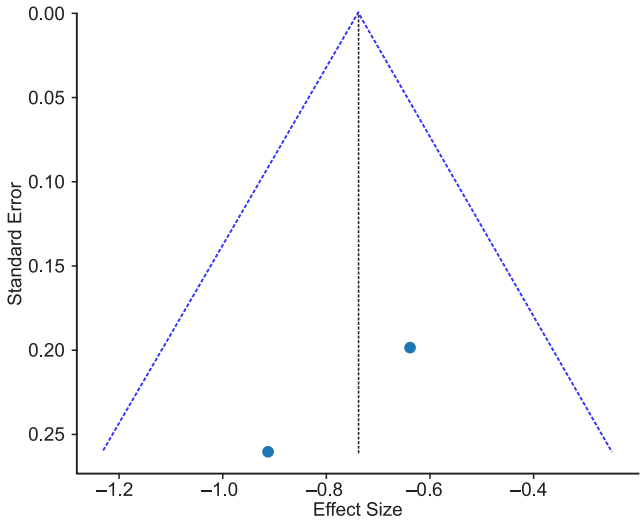

FIG. 15.
Funnel plot of evaluation of the systematic error of publications by the average duration of inpatient treatment after vacuum-assisted laparostomy and other variants of laparostomy without vacuum

nificantly less than that for other laparostomy variants without vacuum ($p = 0.000$).

A symmetrical funnel plot indicates the absence of obvious systematic publication errors (no publication bias).

7. Comparison of vacuum-assisted laparostomy with other multistage surgical treatment options for postoperative mortality.

A. Comparison with on-demand relaparotomy: 2 studies [3, 14] (Fig. 16).

In the presented studies, mortality after vacuum-assisted laparostomy – 18.1 % (34/188) (95% CI: 13.2–24.24 %), after on-demand relaparotomies – 34,64 % (53/153) (95% CI: 27.55–42.48 %).

The relative risk was 0.37 (95% CI: 0.12–1.19; $I^2 = 73.5$ %; random effects model).

In the studies under consideration, **postoperative mortality after vacuum-assisted laparostomy does not** significantly **differ from that after on-demand relaparotomies** ($p = 0.097$).

B. Comparison with programmed relaparotomy: 2 studies (Fig. 17).

In the presented studies [23, 27], postoperative mortality after vacuum-assisted laparostomy – 41.66 % (5/12) (95% CI: 19.26–68.11 %), after programmed relaparotomy – 34.61 % (9/26) (95% CI: 19.31–53.88 %).

The study of N.Y. Patel et al. [48] does not provide any information on postoperative mortality.

The relative risk was 1.58 (95% CI: 0.18–13.83; $I^2 = 55.68$ %; random effects model).

In the studies under consideration, **postoperative mortality after vacuum-assisted laparostomy does not** significantly **differ from that after programmed relaparotomies** ($p = 0.681$).

C. Comparison with other variants of laparostomy without vacuum: 6 studies [9, 13, 20, 29, 47, 49] – 7 comparison groups (Fig. 18, 19).

In the presented studies, postoperative mortality after vacuum-assisted laparostomy – 21.16 % (80/378) (95% CI: 17.34–25.57 %), after other variants of laparostomy without vacuum – 31.51 % (104/330) (95% CI: 26.74–36.72 %).

The relative risk was 0.72 (95% CI: 0.56–0.93; $I^2 = 0$ %; fixed effects model).

In the studies under consideration, **postoperative mortality is** statistically significantly **lower after vacuum-assisted laparostomy in comparison with other variants of laparostomy without vacuum** ($p = 0.012$).

Study ID	Experiment Group		Control Group	
	event	number	event	number
I.B. Uvarov, 2022	3	52	24	78
M.S. Bleszynski, 2016	31	136	29	75

RR, MH, Random

Study ID	*n*	Effect (95% CI)	Weight (%)
I.B. Uvarov, 2022	130	0.19 [0.06, 0.59]	39.89
M.S. Bleszynski, 2016	211	0.59 [0.39, 0.90]	60.11
Total	**341**	**0.37 [0.12, 1.19]**	**100.00**

2 studies included (*N* = 341)
Heterogeneity: Tau^2 = 0.540, Q = 3.77 (*p* = 0.052), I^2 = 73.5 %
Overall effect test: z = 1.66, *p* = 0,097

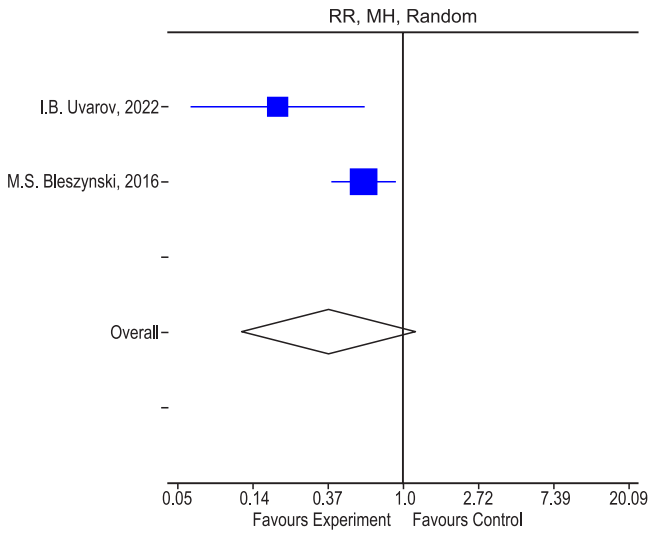

FIG. 16.
Forest plot showing the results of comparing vacuum-assisted laparostomy and relaparotomy "on demand" by postoperative mortality

Study ID	Experiment Group		Control Group	
	event	number	event	number
A.Yu. Anisimov, 2017	4	8	9	14
M.V. Pogorelov, 2020	1	4	0	12

RR, MH, Random

Study ID	*n*	Effect (95% CI)	Weight (%)
A.Yu. Anisimov, 2017	22	0.78 [0.35, 1.72]	69.30
M.V. Pogorelov, 2020	16	7.80 [0.38, 161.43]	30.70
Total	**38**	**1.58 [0.18, 13.83]**	**100.00**

2 studies included (*N* = 38)
Heterogeneity: Tau^2 = 1.604, Q = 2.26 (*p* = 0.142), I^2 = 55.68 %
Overall effect test: z = 0.41, *p* = 0,681

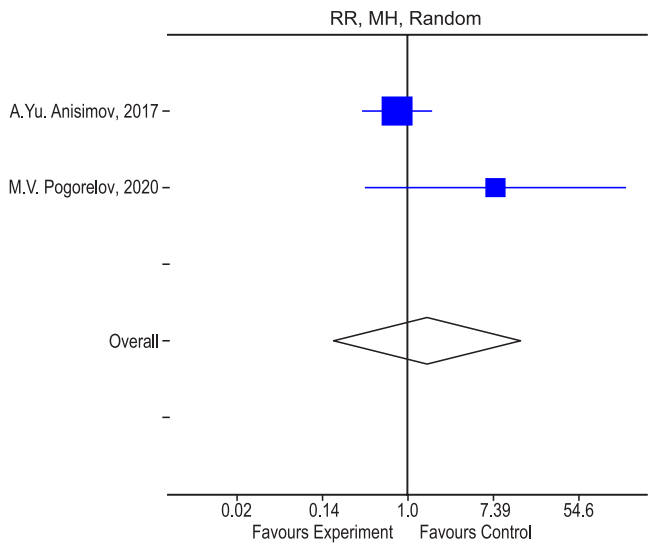

FIG. 17.
Forest plot showing the results of comparing vacuum-assisted laparostomy and programmed relaparotomy by postoperative mortality

The symmetrical funnel plot indicates the absence of obvious systematic publication errors (no publication bias).

DISCUSSION

It is difficult to disagree with the conclusion made by A.V. Sazhin et al. (2020) in their article [7]: "...To date, there are still no clear criteria that allow a practical surgeon to determine the tactics of surgical treatment of widespread peritonitis in a particular patient. A variety of peritoneal inflammation sources, clinical variants of the course of widespread peritonitis and clinical and laboratory changes, combined with the frequent need to use combinations of various treatment methods during surgery and in the postoperative period, explain the need for comprehensive studies that allow to objectively evaluate the results of a particular surgical treatment of peritonitis." A proper choice of a staged surgical treatment in severe and hemodynamically unstable patients having widespread peritonitis, abdominal trauma combined with compartment syndrome, peritonitis, sepsis can significantly improve their treatment outcomes [1, 2].

In our study, we aimed to compare all three methods of multistage management of such patients, given

Study ID	Experiment Group		Control Group	
	event	number	event	number
V.M. Mutafchiyski, 2016	14	49	31	59
T.K. Bee, 2008	8	31	5	20
S. Batacchi, 2009	8	35	11	31
D.V. Cherdantsev, 2016	6	26	14	30
F. Coccolini, 2017	23	163	20	117
F. Coccolini, 2017	11	47	9	42
I. Pliakos, 2012	10	27	14	31

RR, MH, Fixed

Study ID	*n*	Effect (95% CI)	Weight (%)
V.M. Mutafchiyski, 2016	108	0.54 [0.33, 0.90]	26.87
T.K. Bee, 2008	51	1.03 [0.39, 2.71]	5.81
S. Batacchi, 2009	66	0.64 [0.30, 1.39]	11.14
D.V. Cherdantsev, 2016	56	0.49 [0.22, 1.10]	12.42
F. Coccolini, 2017	280	0.83 [0.48, 1.43]	22.24
F. Coccolini, 2017	89	1.09 [0.50, 2.37]	9.08
I. Pliakos, 2012	58	0.82 [0.44, 1.53]	12.45
Total	**708**	**0.72 [0.56, 0.93]**	**100.00**

7 studies included (*N* = 708)
Heterogeneity: Q = 4.16 (*p* = 0.655), I^2 = 0 %
Overall effect test: z = 2.52, *p* = 0,012

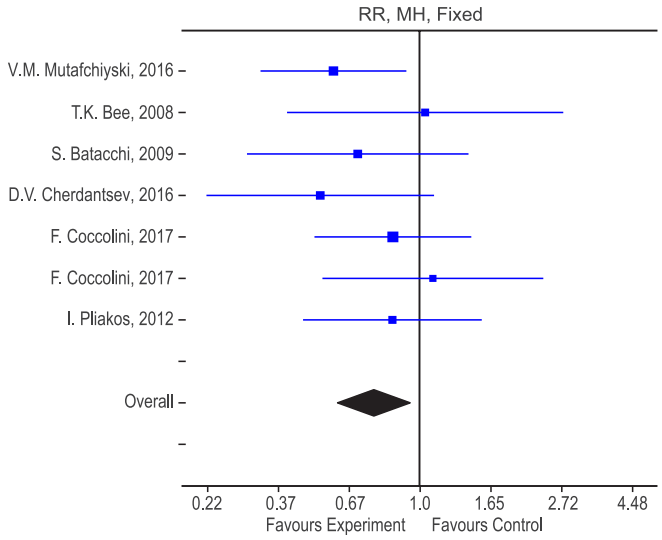

FIG. 18.
Forest plot showing the results of comparing vacuum-assisted laparostomy and laparostomy without vacuum by postoperative mortality

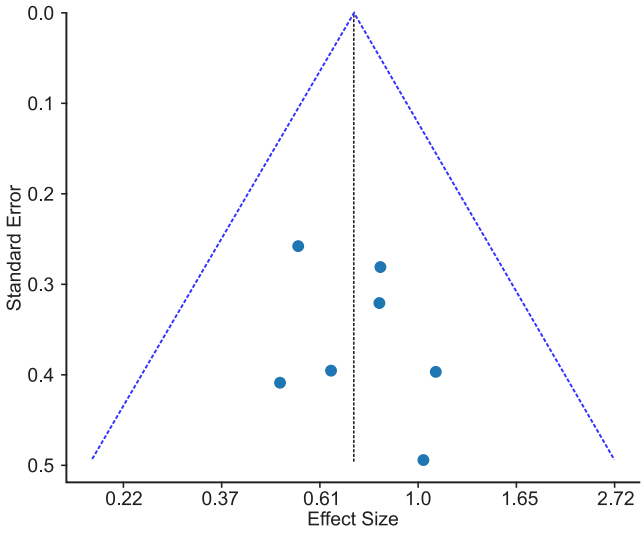

FIG. 19.
Funnel plot of evaluation of the systematic error of publications by postoperative mortality after vacuum-assisted laparostomy and other variants of laparostomy without vacuum

that such studies have not been conducted before. Indications for this or that method of repeated surgical intervention have not yet been clearly given. It is believed that on-demand relaparotomy is indicated in the case of a complication requiring surgery, with negative dynamics of the patient for whom, during the initial surgery for peritonitis or abdominal trauma, there was no need for repeated planned interventions on the abdominal cavity. If the mode of programmed staged re-operations is selected within the framework of *source control* or *damage control* strategies due to the severity of peritonitis, abdominal trauma or the severity of the patient's condition, the surgeon (during the first intervention) determines the need for repeated operations after a certain time interval. Laparostomy technique or "open abdomen" technique implies a negative dynamics in the patient's condition in the form of an increase in intra-abdominal pressure when trying to reduce the edges of aponeurosis and skin and therefore requires temporary closure of the laparotomy wound in other ways. The indications for programmed relaparotomy and laparostomy are nor dissimilar, but it is the presence of IAHS that requires the performance of "open abdomen" technique. The technique of vacuum-assisted laparostomy is promising, but not without its drawbacks. Discussions continue regarding the timing of laparostomy, especially in severely traumatized patients and patients with peritonitis, pancreonecrosis, how to avoid the formation of a "frozen abdomen", methods of final wound closure, complications associated with the possible use of vacuum, advantages and disadvantages of a particular laparostomy technique, expediency and method of reapproximation (medializations) of the edges of a laparotomy wound, and certainly about mortality.

In 2015, in an article by F. Coccolini et al. [10] "The open abdomen, indications, management and definitive closure", published in the World Journal of Emergency Surgery, it was noted that the primary closure of the laparotomy wound during laparostomy is most appropriate in the first 7–8 days after the start of using this technique, and then, in 2017, F. Coccolini et al. [28] specify that this is indicated in the case of elimination of the source of peritonitis, hypoperfusion, when the patient is hemodynamically stable, and when there is no risk of IAHS development or its progression. Subsequently,

the probability of both purulent-septic complications and complications associated with intra-abdominal hypertension increases, and either this requires the use of implants to close the defect of the anterior abdominal wall, or healing takes place by secondary tension with the expected formation of a postoperative hernia and its subsequent surgical treatment [19, 29].

J.J. Atema et al. (2015) [51] in their systematic review and meta-analysis of "open abdomen" in non-traumatized patients (74 studies, 1 RCT, 4,358 patients). MPI averaged 24–34 points, APACHE II – 12–30 points, and the average frequency of successful muscular-aponeurotic closure was 50.2 % (95% CI: 43.4–57.0 %; p (χ^2) < 0.001; I^2 = 90 %), with successful closure being statistically significantly more often observed with laparostomy wound medialization and vacuum techniques, less often with mesh without vacuum and "zip" techniques. The average frequency of enterocutaneous fistula formation was 12.1 % (95% CI: 10.1–14.4 %; p (χ^2) < 0.001; I^2 = 67 %); the highest rate (17.2 %) was observed with the use of mesh, the lowest (5.7 %) – with vacuum laparostomy with wound medialization. Mortality was 30 % (95% CI: 27.1–33.0 %; p (χ^2) < 0.001; I^2 = 69 %).

A.E. Sharrock et al. (2016) [11] conducted a systematic review and meta-analysis on the management of "open abdomen" in traumatized patients as part of *damage control* tactics (26 studies, 1,341 patients) and obtained the following results: the severity of injuries as per the ISS Scale ranged from 19 to 37 points; mortality was 6.07 % (95% CI: 2.61–9.52 %); average laparostomy closure time was 6.62 days (95% CI: 5.44–7.81); average hospital stay duration was 18.57 days (95% CI: 5.150–31.981); overall complication rate was 19.99 % (95% CI: 13.49–26.49 %), with a complication rate of 16.74 % (95% CI: 9.70–23.77 %) for early abdominal closure and 40.85 % (95% CI: 27.90–53.80 %) for late, mesh-assisted closure.

A. Cristaudo et al. (2017) [52] conducted a systematic review and meta-analysis on the analysis of complications and mortality among patients with "open abdomen" (228 studies, 6 RCTs, 13,650 patients). The average frequency of successful muscular-aponeurotic closure was 55 % (95% CI: 52–59 %), and it was also more frequent with laparostomy wound medialization and vacuum techniques (76 %); mortality was 27 % (95% CI: 25–29 %). The average frequency of enterocutaneous fistula formation was 8.5 % (95% CI: 7.4–9.7 %); when comparing vacuum laparostomy and vacuum combined with wound medialization techniques, their number was statistically significantly lower in the second case. The frequency of intra-abdominal abscesses – 13 % (95% CI: 11–16 %); the frequency of postoperative hernias – 15 % (95% CI: 12–19 %) [52].

F. Coccolini et al. (2017) [28] with the grade of recommendations and reliability of the evidence B1 did not recommend the management of laparostomy without negative pressure therapy – only with a mesh or a Bogota bag [28]. 3,125 patients with "open abdomen" were analyzed, 1,942 (62 %) managed to achieve early (within 4-7 days) muscular-aponeurotic closure, which was a factor reducing mortality (12.3 % vs. 24.8 %; RR = 0.53; p < 0.0001) and complication rate (RR = 0.68; p < 0.0001) [28]. Patients with abdominal sepsis are less prone to early muscular-aponeurotic closure, therefore, closure attempts should be made as early as possible after severe abdominal sepsis has been stopped [28].

In a joint study of the S.M. Kirov Military Medical Academy (St. Petersburg) and St. Petersburg Scientific Research Institute of Emergency Care named after I.I. Dzhanelidze, it was noted that the use of VAC-therapy in the treatment of secondary peritonitis reduced mortality from 59 % to 14 %, the frequency of complications in the form of fistula formation – from 7 % to 2.6 % (cit. according to [53]). With IAHS, the use of the method allows primary muscle-aponeurotic closure in 78 % of cases compared to 12.5 % achieved using classical treatment methods (cit. according to [53]).

The development of laparostomy technologies is nonstop. In the first decade of the XXI century, the vacuum-instillation technique of laparostomy management began to be widely used, and the evaluation of the results continues to this day [6]. V.A. Shapkina (2017) [6] evaluated the effectiveness of VAC-laparostomy in combination with fractional flow-instillation technique (n = 25) and without it (n = 24) in the treatment of widespread peritonitis in groups of patients comparable in initial severity (SAPS ≥ 8), MSF severity (SOFA ≥ 12) and severity of abdominal organ diseases (MPI ≥ 20; abdominal index ≥ 13). The author has shown a more rapid decrease in intoxication, normalization of the abdominal cavity, fewer complications (52 % vs. 72 %; fistulas – 47 % vs. 62 %; suppuration of the postoperative wound – 18 % vs. 23 %) and lower mortality (20.8 % vs. 26 %) in the vacuum-instillation laparostomy group [6].

At the same time, D.V. Cherdantsev et al. (2018) [54] published their experience of using vacuum-instillation laparostomy. The study included 47 patients with widespread purulent peritonitis who were divided into 2 groups: the first – 23 patients who had a standard vacuum-assisted laparostomy with the use of VivanoTec device (Hartmann, Germany) in constant vacuuming mode with negative pressure of 120 mm Hg; the second – 24 patients who had vacuum-instillation laparostomy in the perioperative period [54] (cit. according to [53]). The conclusions were made that there were no reliable data on mortality reduction when using vacuum-instillation laparostomy compared to vacuum-assisted laparostomy [54] (cit. according to [53]).

The emergence of systems and methods for wound medialization (ABRA system, abdominal reapproximation anchor system; VAWCM, vacuum-assisted wound closure and mesh-mediated fascial traction, etc.) has improved the treatment results of patients with "open abdomen" in terms of reduced time and increased proportion of primary closure of the abdominal cavity, fewer complications, in particular fistulas, and mortality, respectively [10, 11, 28, 51, 52].

According to many authors, the use of VAC-therapy in the treatment of secondary peritonitis can reduce

the number of repeated lavage relaparotomies, shorten the time of laparotomy wound closure, and reduce the risks of postoperative complications [28, 29, 53]. In our study, we have not obtained such data: on the contrary, when using programmed relaparotomy tactics, statistically significantly fewer repeated interventions were required.

According to the 2017 IROA data, the VAC-laparostomy technique has the lowest mortality rate in comparison with other types of laparostomy without vacuum [9], but ranks second in the risk of enterocutaneous fistula formation [7, 28, 29]. We have not obtained statistically significant differences in the frequency of enterocutaneous fistula occurrence between vacuum-assisted laparostomy and laparostomy without vacuum. At the same time, it can be stated that in terms of length of ICU stay, duration of inpatient care and, most importantly, mortality, statistically significantly better results were obtained while using vacuum than in "open abdomen" without vacuum. And if these differences are not associated with postoperative complications, the timing of the final closure of the abdominal cavity and the number of patients who managed to close the abdominal cavity, it is likely to be due to faster relief of IAHS when using vacuum, given that in the compared cohorts the patients were comparable in severity of condition, severity of peritonitis, hemodynamic parameters.

Obviously, like all meta-analysis, ours has significant limitations. We have found only one RCT (of low quality) comparing the results of VAC-laparostomy with other variants of laparostomy without vacuum; the others are presented by prospective, retrospective and combined cohort studies. There has been a restriction on the search for non-randomized studies since 2007; there has been no such restriction for RCTs. Only full-text articles without language restrictions have been used. Not all studies clearly differentiated by subgroups of patients with abdominal trauma, peritonitis, and other intra-abdominal conditions that caused the transition to the multistage treatment tactics, and, accordingly, the conclusions were not differentiated by these subgroups. We have not aimed to compare different methods of vacuum-assisted laparostomy with each other, but only evaluated possible advantages of vacuum over other technologies of multistage treatment without vacuum aspiration.

CONCLUSION

The results of comparing VAC-laparostomy with other multistage treatment options during our meta-analysis are summarized in Table 2.

The findings of this meta-analysis are as follows:

1. There are no statistically significant differences between vacuum-assisted laparostomy and on-demand relaparotomy in the number of necessary reoperations ($p = 0.319$). The level of evidence (LE) 2, the grade of recommendations (GR) B in accordance with the recommendations of the Center for Healthcare Quality Assessment and Control of the Ministry of Health of the Russian Federation [31].

TABLE 2

RESULTS OF COMPARISON OF VACUUM-ASSISTED LAPAROSTOMY WITH OTHER OPTIONS FOR MULTISTAGE TREATMENT

Methods compared with VAC-laparostomy	Comparison parameters						
	Number of repeated operations required	Number of patients with successful abdominal closure	Average timing of the final closure of the abdominal cavity	Postoperative complications	Average duration of patients' stay in the ICU	Average length of stay in hospital	Postoperative mortality
On-demand relaparotomy	$p = 0.319$	no data	no data	no data	$p = 0.889$	$p = 0.749$	$p = 0.097$
Programmed relaparotomy	**$p = 0.023$** [23, 27]	no data	no data	no data	no data	no data	$p = 0.681$
Laparostomy without vacuum	$p = 0.053$	$p = 0.333$	$p = 0.192$	$p = 0.371$ $p = 0.250$	**$p = 0.000$** [9, 20]	**$p = 0.000$** [20, 62]	**$p = 0.012$** [9, 13, 20, 29, 47, 49]

2. The number of required reoperations with programmed relaparotomies is statistically significantly lower than with vacuum-assisted laparostomy (RR = 2.94 (95% CI: 1.16–7.44); $p = 0.023$). LE 2, GR B [31].

3. There are no statistically significant differences in the number of required reoperations with vacuum laparostomy and laparostomy without vacuum ($p = 0.053$). LE 2, GR B [31].

4. There are no statistically significant differences in the number of patients with successful closure of the abdominal cavity after vacuum-assisted laparostomy or other variants of laparostomy without vacuum ($p = 0.333$). LE 1, GR C [31].

5. The average time of final closure of the abdominal cavity after vacuum-assisted laparostomy and other variants of laparostomy without vacuum do not differ statistically significantly ($p = 0.192$). LE 2, GR B [31].

6. There are no statistically significant differences between vacuum-assisted laparostomy and other variants of laparostomy without vacuum in the frequency of enterocutaneous fistula occurrence ($p = 0.371$). LE 2, GR B [31].

7. There are no statistically significant differences between vacuum-assisted laparostomy and other variants of laparostomy without vacuum in the frequency of intra-abdominal abscesses ($p = 0.250$). LE 2, GR B [31].

8. The average duration of stay in the ICU after vacuum-assisted laparostomy and on-demand relaparotomy do not differ statistically significantly ($p = 0.889$). LE 2, GR B [31].

9. The average duration of treatment in the ICU after vacuum-assisted laparostomy is statistically significantly shorter than with laparostomy without vacuum (SMD = –0.66 (95% CI: –0.96÷–0.35); $p = 0.000$). LE 2, GR B [31].

10. The average duration of inpatient treatment after vacuum-assisted laparostomy and on-demand relaparotomy does not differ statistically significantly ($p = 0.749$). LE 2, GR B [31].

11. The average duration of inpatient treatment after vacuum-assisted laparostomy is statistically significantly shorter than with laparostomy without vacuum (SMD = –0.74 (95% CI: –1.05÷–0.43); $p = 0.000$)). LE 2, GR B [31].

12. Postoperative mortality after vacuum-assisted laparostomy does not significantly differ from that after on-demand relaparotomies ($p = 0.097$). LE 2, GR B [31].

13. Postoperative mortality after vacuum-assisted laparostomy does not significantly differ from that after programmed relaparotomy ($p = 0.681$). LE 2, GR B [31].

14. Mortality after vacuum-assisted laparostomy is statistically significantly lower in comparison with other types of laparostomy without vacuum (HR = 0.72 (95% CI: 0.56–0.93); $p = 0.012$). LE 1, GR C [31].

KEY TAKEAWAYS

Based on the results of national and international studies and our meta-analysis, we can point out the validity and effectiveness of vacuum-assisted laparostomy in the treatment of severe abdominal trauma and urgent abdominal pathology with compartment syndrome, peritonitis and sepsis. The technique has a number of advantages over other types of laparostomy without vacuum. We also want to emphasize the expediency of further use of on-demand relaparotomies and programmed relaparotomies with appropriate indications.

Further systematic reviews and meta-analyses based on randomized clinical trials with a well-designed model and high-quality methodology are necessary to provide a higher level of evidence and grade of recommendations.

Conflict of interest

The authors of this article declare no conflict of interest.

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VIDEOLAPAROSCOPIC SURGICAL TREATMENT OF STRANGULATED PARAESOPHAGEAL HERNIA (CLINICAL OBSERVATION)

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ABSTRACT

Background. *Paraesophageal hernias are much less common than other types of diaphragmatic hernias. The risk of strangulation in this pathology is about 21 %. It is often very difficult to establish the correct diagnosis in time, because patients are admitted with an atypical clinic of acute cardiological or pulmonary pathology. Usually, the correct diagnosis is established only on the 4th day. Due to late diagnosis, necrosis and perforation of the strangulated organ occur, which causes high mortality; sometimes the correct diagnosis is established only at autopsy.*

Clinical observation. *The patient was admitted to the surgical department on an emergency basis with complaints of intense pain in the lower chest and epigastric region, vomiting of eaten food. From the anamnesis it is known that about a year ago, periodic nausea and vomiting of food eaten began to bother. The patient had been experiencing increased chest and epigastric pain and vomiting for the last 12 hours before admission to the hospital. X-ray diagnosed strangulated paraesophageal hernia of the esophageal opening of the diaphragm. During esophagogastroduodenoscopy it was impossible to pass into the distal parts of the stomach; hyperemia and petechial hemorrhages were detected in the zone of strangulation. Video-laparoscopic reduction of the hernial contents, resection of the hernial sac and anterior diaphragm crurorrhaphy were urgently performed. The early postoperative period was uneventful. The presented clinical observation indicates the promise of using video-endoscopic technologies for diagnosis and treatment of strangulated diaphragmatic hernias. The key to success is the timely establishment of the correct diagnosis. We consider it impractical to perform an antireflux intervention simultaneously in conditions of an acute inflammatory process.*

Key words: *strangulated paraesophageal hernia, video-laparoscopic surgery, gastroesophageal hernia, diaphragm cruraphy*

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ВИДЕОЛАПАРОСКОПИЧЕСКОЕ ОПЕРАТИВНОЕ ЛЕЧЕНИЕ УЩЕМЛЁННОЙ ПАРАЭЗОФАГЕАЛЬНОЙ ГРЫЖИ ПИЩЕВОДНОГО ОТВЕРСТИЯ ДИАФРАГМЫ (КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ)

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РЕЗЮМЕ

Актуальность. *Параэзофагеальные грыжи пищеводного отверстия диафрагмы встречаются значительно реже других типов диафрагмальных грыж. Риск ущемления при этой патологии составляет около 21 %. Зачастую очень сложно своевременно установить верный диагноз, поскольку пациенты поступают с нетипичной клиникой острой кардиологической или пульмонологической патологии. Как правило верный диагноз устанавливается только на 4-е сутки. Из-за поздней диагностики возникают некрозы и перфорации ущемлённого органа, что вызывает высокую летальность. Иногда правильный диагноз устанавливается только при патологоанатомическом вскрытии.*

Клиническое наблюдение. *Пациентка поступила в хирургическое отделение в экстренном порядке с жалобами на интенсивные боли в нижних отделах грудной клетки и эпигастральной области, рвоту съеденной пищей. Из анамнеза известно, что около года назад стали беспокоить периодические тошнота и рвота съеденной пищей. Последние 12 часов до поступления в стационар усилились боли в грудной клетке и эпигастрии, вся съеденная пища вышла со рвотой. Рентгенологически диагностирована ущемлённая параэзофагеальная грыжа пищеводного отверстия диафрагмы. При фиброэзогастродуоденоскопии пройти в дистальные отделы желудка невозможно, в зоне ущемления определяется гиперемия и петехиальные кровоизлияния. В экстренном порядке выполнено видеолапароскопическое вправление грыжевого содержимого, резекция грыжевого мешка и передняя диафрагмокрурорафия. Ранний послеоперационный период протекал без осложнений. Представленное клиническое наблюдение указывает на перспективность использования видеоэндоскопических технологий в диагностике и лечении ущемлённых диафрагмальных грыж. Ключевым моментом успеха является своевременная постановка верного диагноза. Считаем нецелесообразным одномоментное выполнение антирефлюксного вмешательства в условиях острого воспалительного перипроцесса.*

Ключевые слова: *ущемлённая параэзофагеальная грыжа, видеолапароскопическая хирургия, грыжа пищеводного отверстия диафрагмы, диафрагмокрурорафия*

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RELEVANCE

Paraesophageal hernia occurs when abdominal organs (stomach, small and large intestine loops, large omentum) moves up to the posterior mediastinum through the esophageal opening of the diaphragm next to the esophagus. Paraesophageal hernias are extremely rare compared with other types of diaphragmatic hernias. Their frequency varies from 0.4 to 1.4 % of cases [1]. The risk of strangulation in case of this pathology is about 21 % [2]. Due to the rarity and complexity of the diagnosis, the average time to determine the correct diagnosis is about 4 days [3]. Patients are often admitted to the emergency room with an atypical manifestation of acute cardiological or pulmonological pathology [4]. While the diagnosis is being made, such formidable complications as perforation and necrosis of the affected organ, acute posterior mediastinitis and pleural empyema can develop. According to different clinics, the mortality rate due to strangulated paraesophageal hernia ranges from 11.1 to 66 % [3–5]. Sometimes the diagnosis can be determined only during a pathoanatomic autopsy (Fig. 1).

FIG. 1.
Strangulated paraesophageal hernia, as the cause of death of the patient, revealed at postmortem autopsy (photo courtesy of the Bureau of Forensic Medical Examination of the Altai Territory): **1** *– strangulating ring;* **2** *– strangulated fundus and body of the stomach;* **3** *– left lung*

There are isolated observations of the successful use of video endoscopic and robot-assisted technologies in the surgical treatment of strangulated paraesophageal hernias [2, 4, 6, 7].

CASE STUDY

A 38-year-old female patient was taken by ambulance to the surgical department in an emergency with complaints of intense pain in the lower half of the chest and epigastrium, nausea and vomiting. It is known from the patient history that she has been in this condition for about a year, when recurrent nausea and vomiting of eaten food occurred. During the last week, the frequency of these manifestations has been increasing, moderate dull epigastric pain occurred. Previously, X-ray examination diagnosed a fixed paraesophageal hernia. The patient had been experiencing increased chest and epigastric pain and vomiting for the last 12 hours before admission to the hospital.

Physical examination data

The patient's condition on admission of moderate severity, conscious, oriented to person, time and place. Normosthenic body type. BMI – 28 kg/m^2 (overweight), body temperature – 36.6 °C. The skin is flesh-colored. The skin is normohydrated, turgor and elasticity are normal. Vesicular respiration during auscultation in all lung fields, no rale. The number of respiratory movements is 19 per minute. The arterial pressure in the brachial arteries is 120/80 mm Hg, same on the left and right. The pulse is 96 bpm. During auscultation of the heart – tones are clear, no murmurs. Status localis: the tongue is moist, covered with white plaque in the back area. When examined, the abdomen is of the correct shape, participates in the act of breathing, there are no deformities of the anterior abdominal wall. An atrophic scar is determined along the midline from the navel to the womb (after cesarean section). No hernia defects of the anterior abdominal wall detected. Deep palpation reveals moderate tenderness in the epigastric region. There is no muscular defense. According to the percussion of the liver (Kurlov's method), size of the liver is 9–8–7 cm. Rebound tenderness (Shyotkin – Blumberg sign) is negative. Auscultation: intestinal motility active. Per rectum: no space-occupying lesions (SOLs) in the rectum palpated, traces of brown stool on the glove.

Results of additional study methods

Laboratory parameters within the reference values (leukocytes – 5.9 × 10^9/L, hemoglobin – 158 g/L). ECG – sinus rhythm 90 per minute, increased cardiac electrical activity. X-ray examination of the oesophagus and stomach – upright position above the diaphragm reveals the cardiac, vault and body of the stomach with pronounced pneumatosis and fluid level above the diaphragm, contrast is administered in small portions to the antral area (Fig. 2).

Fibro-esogastroduodenoscopy – gastric contents were found to be thrown from the stomach into the oesophagus, there was a small amount of frothy fluid in the stomach, mucosa of the stomach fundus and body had diffuse hyperemia and petechial haemorrhages, it was impossible to go into the antral area as a result of the anatomic position of the stomach. In view of the intense pain syndrome, the clinical picture of high gastrointestinal obstruction and the radiological picture of a fixed paraesophageal hernia, the diagnosis has been established as a strangulated paraesophageal hernia.

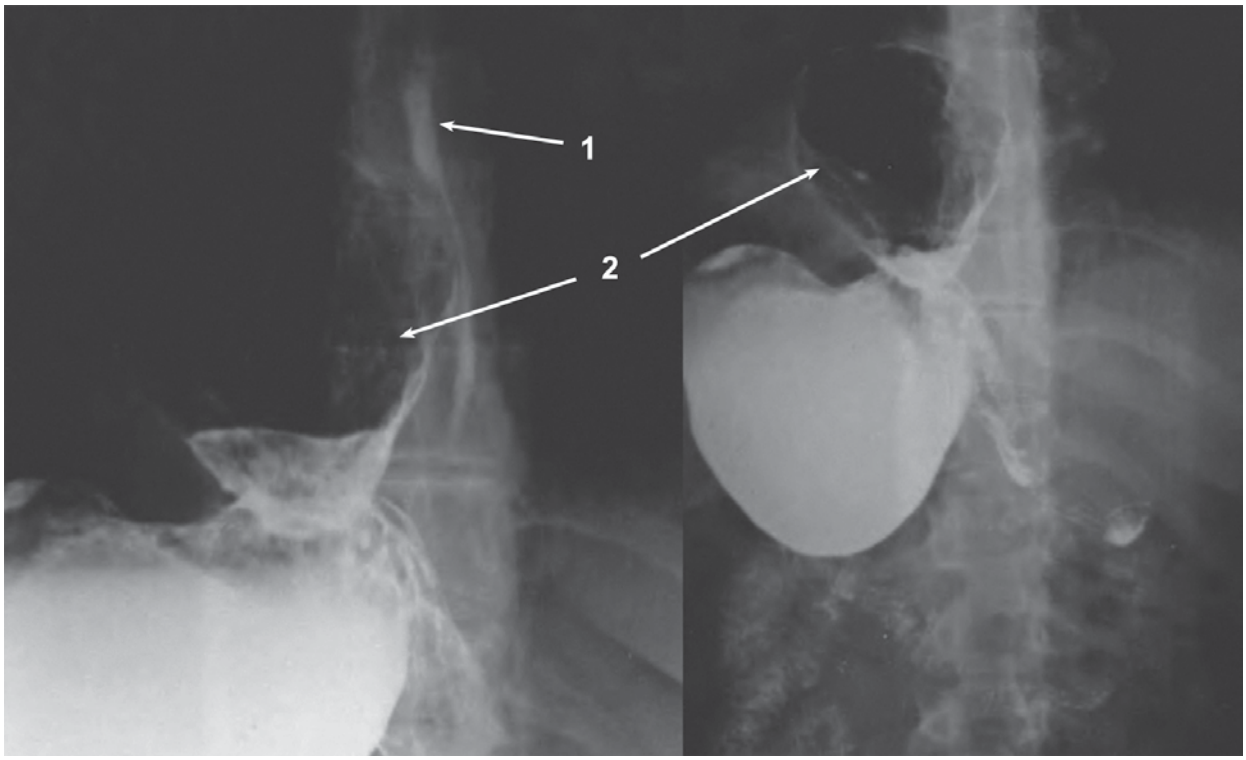

FIG. 2.
X-ray of the patient with a contrast examination of the esophagus and stomach after admission to the clinic (direct projection): **1** *– esophagus;* **2** *– strangulated part of the stomach*

Treatment policy

An emergency operation was performed under general anaesthesia 2 hours and 30 minutes after admission to the hospital. A 10 mm trocar is implanted in the mesogastric area (8 cm above the umbilicus). CO_2 insufflation up to 12 mmHg. There is no effusion in the abdominal cavity, the hypogastrium has omental adhesions to the anterior abdominal wall, the parietal and visceral peritoneum is smooth and shiny. The liver is enlarged due to the left lobe, its surface is smooth, red-brown in color, the edge is rounded. The stomach is not enlarged and the visible part of the anterior wall is unchanged. Visible loops of the small and large intestine without organic pathology. The oesophageal opening of the diaphragm is dilated to 5 cm. The fundus, the body of the stomach and the strand of the large omentum are located in the posterior mediastinum (Fig. 3).

The subcostal areas have 10 mm (left) and 5 mm (right) trocars for introducing the operating surgeon's instruments; two 5 mm trocars are implanted subxiphoidally and along the left lateral abdominal wall for the first surgical assistant. The stomach is grasped by an atraumatic grasper and is lowered into the abdominal cavity in a pendulum-like, stepwise movements with a slight tension. The omentum and the strangulated part of the stomach are moderately hyperemic, viable. Tissues in the area of the esophageal hiatus with signs of inflammatory process with scarring (Fig. 4).

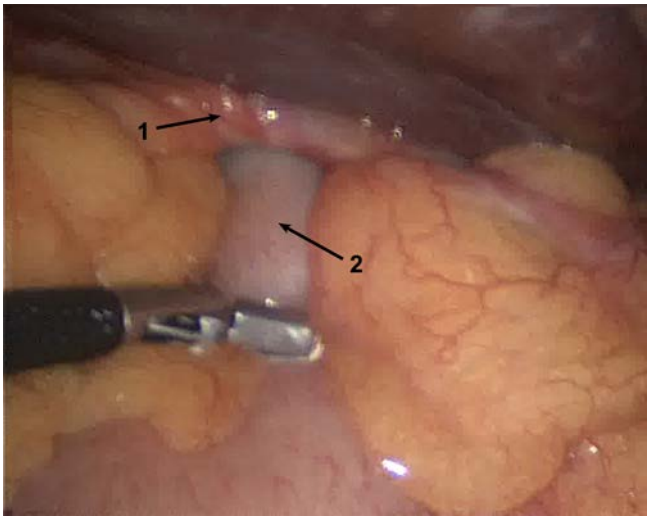

FIG. 3.
Intraoperative photography during video-laparoscopy of the patient: **1** *– the esophageal opening of the diaphragm;* **2** *– the stomach strangled in the posterior mediastinum*

There is up to 20 ml of hernial water of brown color in the hernial sac; the walls are also hyperemic (Fig. 5). A 32 F gastric tube has been inserted into the stomach through the mouth. The gastric contents on the tube are scanty and have no blood in them. Using an ultrasound dissector,

the herniated sac was dissected laterally and medially around the circumference of the oesophageal opening, bluntly isolated in the posterior mediastinum, and dissected off. The crura of diaphragm are highlighted anterior to the oesophagus (Fig. 6). The cardia area is 2 cm below the oesophageal opening. The crura are sutured in front of the oesophagus with a Z-shaped stitch (Fig. 7). The esophageal opening of the diaphragm is narrowed to 2 cm. Hemostasis – no bleeding.

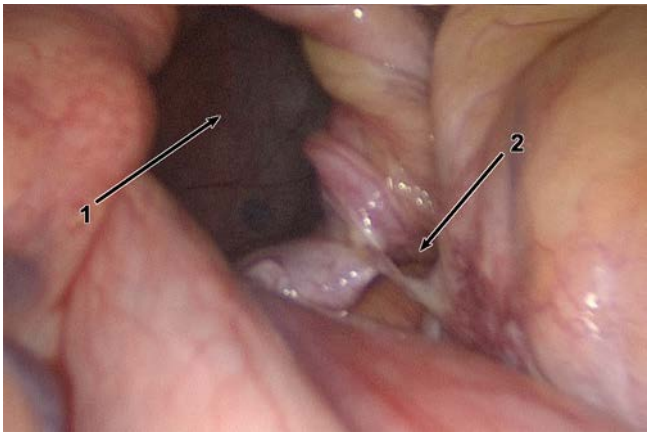

FIG. 4.
Intraoperative photography during video-laparoscopy of the patient after repositioning the hernial contents into the abdominal cavity: **1** *– esophageal opening of the diaphragm;* **2** *– scar-inflammatory process*

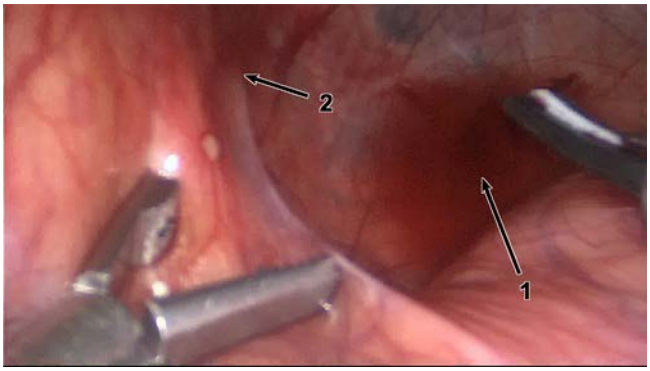

FIG. 5.
Intraoperative photography with video-laparoscopy of the hernial sac: **1** *– hernial water with a hemorrhagic component;* **2** *– catarrhal inflammation of the walls of the hernial sac*

A latex drainage tube is implanted in the left subhepatic space. Histological conclusion – hernial sac with signs of acute inflammation. Post-operative diagnosis – para-esophageal hemigastric gastroesophageal hernia of the oesophageal diaphragm opening type II, grade 3, complicated by impingement.

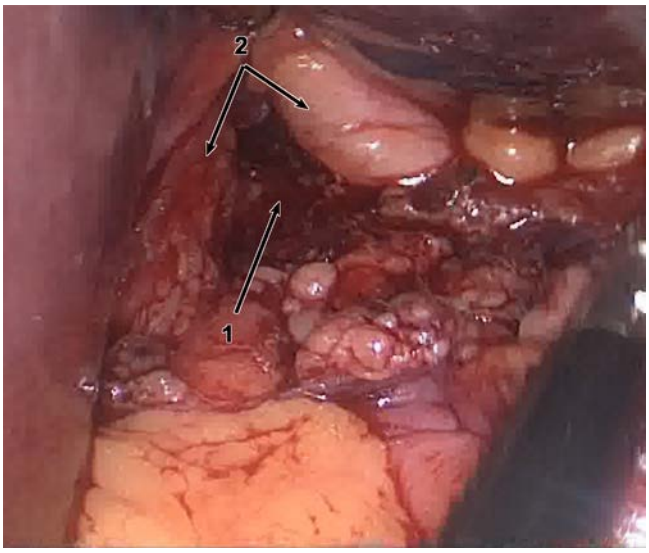

FIG. 6.
Intraoperative photography during video-laparoscopy of the patient after exposure of the diaphragm crus: **1** *– the esophagus;* **2** *– the crus of the diaphragm*

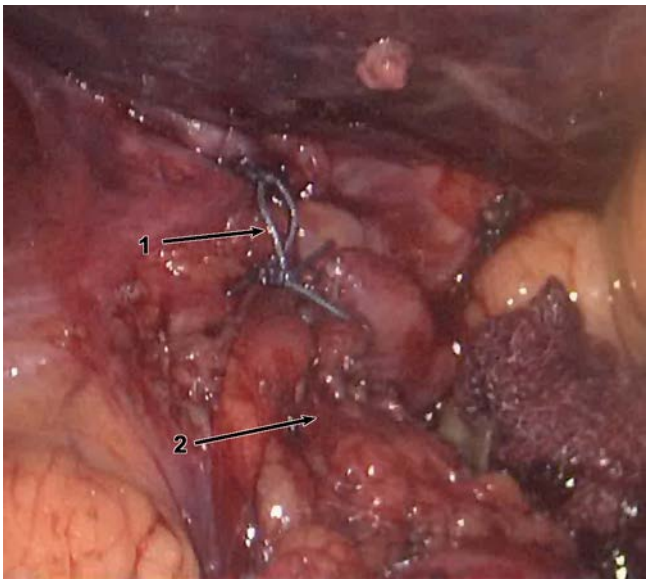

FIG. 7.
Intraoperative photography during video-laparoscopy of the patient after anterior diaphragm cruraphy: **1** *– esophagus;* **2** *– sutured crus of the diaphragm*

Treatment outcome

After the surgery the patient received injections of analgesics (ketoprofen), antibiotics (ceftriaxone) and anticoagulants (heparin). No complications were diagnosed in the early postoperative period. Postoperative complete blood count without inflammatory changes (leukocytes 6.4 × 10^9/l), slight increase in liver enzymes (AST – 136.3 U/l; ALT – 76.8 U/l) and mild hyperbilirubinemia (total – 22.5 μmol/l, indirect – 14.2 μmol/l).

Other laboratory parameters – without pathological changes. On the 4th day after the surgery, the esophagus and stomach were radiographed: the stomach is located below the diaphragm, the cardia is freely passable, no contrast agent was detected outside the oesophagus and stomach contour (Fig. 8). At the 10th day, the patient was discharged from hospital, with a recommendation for a follow-up examination in the surgical department to decide whether esophagofundoplasty should be performed as a routine procedure.

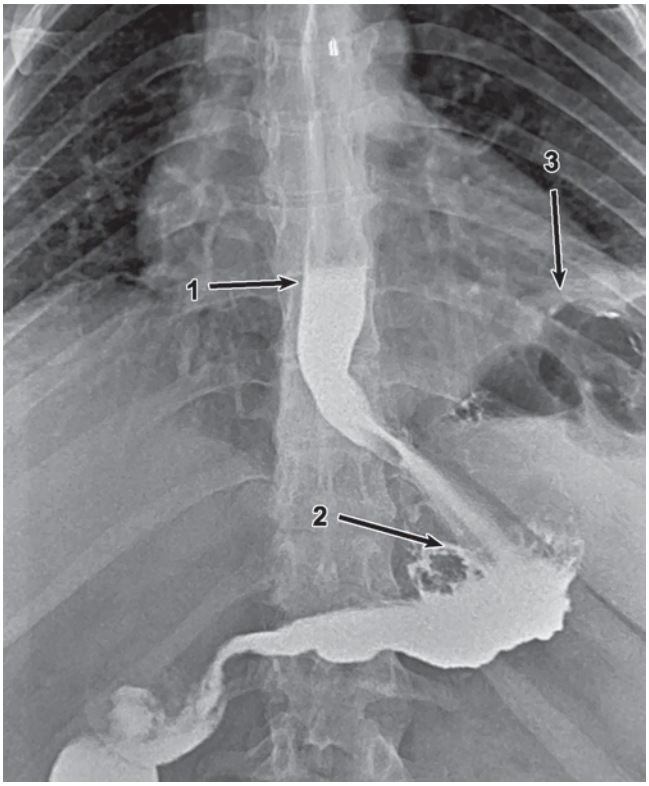

FIG. 8.
X-ray of the patient with contrast examination of the esophagus and stomach on the 4th day after surgery (direct projection): 1 – esophagus; 2 – stomach; 3 – diaphragm

CONCLUSION

The case study demonstrates the possibility of effective use of video endoscopic technologies in treatment of strangulated paraesophageal hernias of the esophageal opening of the diaphragm. The key element of success, in our opinion, was the timely diagnosis based on the patient's medical history. The limitation of the surgical volume by performing the reduction of hernial contents with resection of the sac and anterior cruroraphy is due to the inflammatory changes in the stomach wall and, accordingly, an increased risk of complications.

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

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RESULTS OF SLEEVE GASTRECTOMY IN OBESE PATIENTS WITH TYPE 2 DIABETES MELLITUS AND IMPAIRED GLUCOSE TOLERANCE: RETROSPECTIVE COHORT REGISTRY-BASED STUDY

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ABSTRACT

Background. *In the available literature, the data on the positive effects of sleeve gastrectomy in treatment of type 2 diabetes mellitus (T2DM) and impaired glucose tolerance (IGT) become more common, however, they are heterogeneous and not always unambiguous.*

The aim. *To analyze our own results of treatment of patients with type 2 diabetes mellitus and impaired glucose tolerance, who underwent sleeve gastrectomy.*

Materials and methods. *Retrospective cohort registry-based study was carried out. From 2016 to April 2021, 29 (19 %) and 7 (4.6 %) patients with diagnosed T2DM and IGT respectively underwent surgery. Of these, sleeve gastrectomy was performed in 13 (44.8 %) patients with type 2 diabetes mellitus and in 5 (71.4 %) patients with IGT. The mean duration of follow-up for T2DM and IGT patients was 14.2 ± 12.3 and 11.2 ± 9.0 months respectively.*

Results. *The mean %EWL (% excess weight loss) in patients with T2DM and IGT was 44.1 ± 17.3 and 51.5 ± 16.9 respectively, and the mean %TWL (% total weight loss) was 25.0 ± 8 and 27.8 ± 6.0 respectively. At the moment of observation, all patients had normal level of fasting blood glucose. The level of HbA1c in patients with type 2 diabetes before the surgery was 8.2 ± 1.6, after surgery, at the time of observation – 5.8 ± 0.5 ($U = 4$; $p \leq 0.01$). Target HbA1c values were recorded in all 13 patients with type 2 diabetes.*

Conclusion. *Our study shows the efficiency of sleeve gastrectomy both in terms of weight loss and of the remission for patients with T2DM and IGT.*

Key words: *bariatric surgery, metabolic surgery, sleeve gastrectomy, type 2 diabetes mellitus, obesity*

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РЕЗУЛЬТАТЫ ПРОДОЛЬНОЙ РЕЗЕКЦИИ ЖЕЛУДКА У ПАЦИЕНТОВ С ОЖИРЕНИЕМ В СОЧЕТАНИИ С САХАРНЫМ ДИАБЕТОМ 2-ГО ТИПА И НАРУШЕНИЕМ ТОЛЕРАНТНОСТИ К ГЛЮКОЗЕ: РЕТРОСПЕКТИВНОЕ КОГОРТНОЕ REGISTRY-BASED ИССЛЕДОВАНИЕ

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РЕЗЮМЕ

Обоснование. *В доступной литературе всё чаще встречаются данные о положительных эффектах продольной резекции желудка в отношении сахарного диабета (СД) 2-го типа и нарушения толерантности к глюкозе (НТГ), однако они разнородны и не всегда однозначны.*

Цель исследования. *Анализ собственных результатов лечения пациентов с сахарным диабетом 2-го типа и нарушением толерантности к глюкозе, которым выполнена продольная резекция желудка.*

Методы. *Дизайн – ретроспективное когортное registry-based исследование. За период с 2016 г. по апрель 2022 г. с диагнозом СД 2-го типа и НТГ было прооперировано 29 (19 %) и 7 (4,6 %) пациентов соответственно. Из них продольная резекция желудка выполнена 13 (44,8 %) пациентам с СД 2-го типа и 5 (71,4 %) пациентам с НТГ. Средняя продолжительность наблюдения за пациентами с СД 2-го типа и НТГ составила 14,2 ± 12,3 и 11,2 ± 9,0 мес. соответственно.*

Результаты. *Средний процент потери избыточной массы тела (%EWL, % excess weight loss) у пациентов с СД 2-го типа и НТГ составил 44,1 ± 17,3 и 51,5 ± 16,9 соответственно, а средний процент потери общей массы тела (%TWL, % total weight loss) – 25,0 ± 8,0 и 27,8 ± 6,0 соответственно. У всех пациентов на момент наблюдения достоверно зафиксирована нормализация уровня гликемии натощак. Уровень HbA1c у пациентов с СД 2-го типа перед операцией – 8,2 ± 1,6, после операции на момент наблюдения – 5,8 ± 0,5 ($U = 4$; $p \leq 0,01$). Достижение целевых значений HbA1c установлено у всех 13 пациентов с СД 2-го типа.*

Заключение. *Наше исследование показывает эффективность продольной резекции желудка как в отношении снижения массы тела, так и в части компенсации СД 2-го типа и НТГ.*

Ключевые слова: *бариатрическая хирургия, метаболическая хирургия, продольная резекция желудка, сахарный диабет 2-го типа, ожирение*

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OBJECTIVES

Bariatric and metabolic surgery is increasingly becoming the method of choice in the treatment of obesity and type 2 diabetes mellitus (T2DM). Numerous studies show statistically significant effectiveness of surgical methods for the treatment of T2DM compared with traditional medical methods [1–3].

The results in complete or partial remission of T2DM between operations are ambiguous. It is believed that the best results of surgical treatment of T2DM are demonstrated by gastric bypass interventions [4, 5].

However, there is increasing evidence on the effectiveness of sleeve gastrectomy in terms of remission of T2DM due to the metabolic effects identified in this procedure [6–9]. Meta-analysis by Y. Han et al. (2020) confirms the similar effectiveness of sleeve gastrectomy in comparison with standard gastric bypass in terms of reducing excess weight and controlling T2DM, separately focusing on the lower frequency of complications and repeated operations after sleeve gastrectomy [10].

Attempts are also being made to stratify the choice of surgery depending on the patient's body mass index (BMI) and the duration of T2DM. For example, Roux-en-Y gastric bypass was the optimal choice for patients with a duration of T2DM > 5 years in terms of achieving complete remission of diabetes 1 year after surgery. Nevertheless, sleeve gastrectomy proved to be a cost-effective choice for patients with a duration of T2DM ≤ 5 years and BMI ≥ 35.5 kg/m^2 [11].

Sleeve gastrectomy is a surgery whose effects are still being actively studied. It can become an option of choice in the case when there is no technical possibility to perform a bypass operation (multiple operations on the abdominal cavity with a massive adhesion), and it is safer to perform sleeve gastrectomy if the patient has a high prevalence of comorbidities. In patients who smoke and do not plan to give up nicotine, a bypass surgery is a risk of ulceration in the gastroenteroanastomosis area. SADIs surgery (single anastomosis duodenal-ileal bypass with sleeve) can solve this problem, but technically it is much more difficult to perform and requires a serious level of compliance from the patient. Here, the patient's unwillingness to constantly take vitamin and mineral complexes can also tip the scales in favor of sleeve gastrectomy.

In this article we analyzed our own results of treatment of patients with type 2 diabetes mellitus and impaired glucose tolerance (IGT), who underwent sleeve gastrectomy.

METHODS

Study design. To achieve this goal, a retrospective cohort registry-based study was carried out. During the study, the National Bariatric Register collected and stored patient observation data.

Compliance criteria. Inclusion criteria: 1) sleeve gastrectomy; 2) type 2 diabetes mellitus or IGT. Exclusion criteria: 1) any other bariatric procedures; 2) no T2DM or IGT at the time of surgery.

Procedure situation. The study was conducted in the elective surgery unit of the Nizhnevartovsk District Clinical Hospital.

Duration of the study. This article presents intermediate data (from 2016 to April 2022), allowing to assess the feasibility of conducting the study in the future.

Description of the medical intervention. The study is based on the experience of surgical treatment of 153 obese patients admitted to the Nizhnevartovsk District Clinical Hospital from 2016 to April 2022. Sleeve gastrectomy was performed in 125 (81 %) patients; MGB-OAGB surgery (mini gastric bypass – one anastomosis gastric bypass) – 20 (13 %); standard bypass surgery – 2 (1 %); SASi bypass surgery (single anastomosis sleeve ileal bypass) – 3 (2 %); gastroplication – 1 (1 %); bariatric revision surgery – 4 (3 %). The treatment of obese patients was carried out in accordance with the National Clinical Recommendations for Morbid Obesity Treatment in Adults (3rd revision) [12].

A total of 29 (19 %) and 7 (4.6 %) patients with T2DM and IGT respectively were operated. Sleeve gastrectomy was performed in 13 (44.8 %) patients with type 2 diabetes mellitus and 5 (71.4 %) patients with impaired glucose tolerance. Demographic data are presented in Table 1.

From a technical point of view, all surgeries were performed in accordance with the clinical practical guidelines of the European Association of Endoscopic Surgery (EAES) for bariatric surgery [13] and included the use of disposable suturing instruments and cartridges, modern surgical energy for tissue separation and ligation. All surgeries are performed using a calibration tube (38 Fr).

All patients were operated under general anesthesia with the use of inhalation anesthetic gas. All patients underwent preoperative antibiotic prevention with cephalosporins of the I generation 30 minutes before surgery. Prevention of venous thromboembolic complications was carried out according to clinical recommendations and included early activation of the patient, which began 6 hours after surgery, compression garment of the lower extremities, the appointment of low molecular heparins in preventive dosages.

In the postoperative period, all patients underwent standardized fluid therapy and analgesia.

Only 2 patients with IGT and 6 patients with T2DM received single-drug preoperative oral antidiabetic therapy. Four patients with T2DM took 2 or more oral antidiabetic drugs. Oral antidiabetic drugs (OAD) in combination with prolonged forms of insulin were received by 2 patients with T2DM. In 1 case, T2DM was corrected for the first time by preoperative diet therapy.

The main outcome of the study: achievement of normoglycemia and target values of HbA1c in patients after surgery at the moment of observation; achievement of target values of weight loss (percentage of excess weight loss (%EWL) ≥ 50; percentage of total weight loss (%TWL) ≥ 25).

Additional outcomes of the study: postoperative bed-day, the number and nature of complications.

Methods of statistical data processing. The data were retrospectively collected in the database of the National Bariatric Register of the Russian Federation and included the period from 2016 to April 2022. Descriptive

TABLE 1
DEMOGRAPHICS INFORMATION OF THE PATIENTS

Parameters	Patients with T2DM (*n* = 13)	Patients with IGT (*n* = 5)
Female	9	2
Mean follow-up duration, months	14.2 ± 12.3 (1–36)	11.2 ± 9.0 (4–21)
Mean age, years	51.1 ± 12.3 (31–71)	42.8 ± 7.1 (37–55)
Mean weight before surgery, kg	127.3 ± 22.3 (86–169)	129.8 ± 21.6 (107–156)
Mean BMI, kg/m^2	45.5 ± 8.3 (32.4–62.9)	43.6 ± 8.2 (32.3–51.4)
History of T2DM and IGT, months	52.8 ± 72.2 (1–240)	10.0 ± 9.6 (1–24)
Mean risk according to ASA	2.6 ± 0.5	2.4 ± 0.5
Micro- and macroangiopathies, *n*	2	0
High-risk AH	9	3
CAD	2	1
OSA	1	1
Hyperlipidemia/dyslipidemia	8	3
NAFLD	8	3
CHF	8	2
Atherosclerosis	3	1
Smoking	7	3

Note. ASA – American Society of Anesthesiologists; AH – arterial hypertension; CAD – coronary artery disease; OSA – obstructive sleep apnea; NAFLD – non-alcoholic fatty liver disease; CHF – chronic heart failure.

analysis included the calculation of mean values, standard deviations and proportions. During the analysis, a non-parametric test (Mann – Whitney U test for numerical data and Pearson Chi-Square test with Yates' continuity correction for relative indicators) was used to evaluate differences in subgroups when calculating *p*-values. The value of $p < 0.05$ was considered a statistically significant difference. All calculations were carried out using a licensed Statistica 13.0 software package for statistical analysis (StatSoft Inc., USA).

RESULTS

The duration of the surgery in patients with T2DM and IGT did not differ statistically significantly (118.1 ± 39.1 and 102.0 ± 26.1 min, respectively; $U = 27$; $p > 0.05$).

The main results of the study. All patients had sustained weight loss after the surgery. The results of weight loss are shown in Table 2.

The variability of values is associated with different duration of patient follow-up: from 1 to 36 months in patients with T2DM and from 4 to 21 months in patients with IGT.

The distribution of patients by blood glucose level before and after the surgery at the moment of observation is shown in Table 3.

As indicated in the table, at the moment of observation all patients had normal level of fasting blood glucose. The blood glucose level in patients with IGT returned to normal after 1.4 ± 0.4 months.

The level of HbA1c in patients with T2DM before surgery was 8.2 ± 1.6, after surgery, at the moment of observation – 5.8 ± 0.5 ($U = 4$; $p \leq 0.01$). Target HbA1c values were recorded in all 13 patients with type 2 diabetes. Complete withdrawal of OAD was achieved in 9 patients, OAD dosage reduction – in 2 patients, reduction of dosages of OAD and prolonged forms of insulin – in 1 patient. Complete withdrawal of insulin therapy with reduced dosages of OAD was registered in 1 patient. Target HbA1c values in patients with T2DM noted within 4.0 ± 1.7 months.

TABLE 2

RESULTS OF WEIGHT LOSS IN PATIENTS WITH TYPE 2 DIABETES AND IMPAIRED GLUCOSE TOLERANCE

Parameters	Patients with T2DM (*n* = 13)	Patients with IGT (*n* = 5)
Mean weight before surgery, kg	127.3 ± 22.3 (86–169)	129.8 ± 21.6 (107–156)
Mean BMI before surgery, kg/m^2	45.5 ± 8.3 (32.4–62.9)	43.6 ± 8.2 (32.3–51.4)
Mean weight after surgery, kg	95.2 ± 17.7 (59–121)	93.8 ± 18.8 (76–117)
Mean %EWL	44.1 ± 17.3 (12.0–79.4)	51.5 ± 16.9 (37.2–73.7)
Mean %TWL	25.0 ± 8.7 (6.6–39.6)	27.8 ± 6.0 (22.4–36.9)
Mean TWL, kg	32.1 ± 14.4 (8–67)	36.0 ± 8.7 (24–48)
Mean TBMIL, kg/m^2	11.5 ± 5.0 (3.0–21.1)	12.3 ± 4.2 (7.2–19.0)

Note. TBMIL – total body mass index loss

TABLE 3

DISPOSITION OF PATIENTS BY THE BLOOD GLUCOSE LEVEL

Patients	Blood glucose level before the surgery, mmol/L	Blood glucose level at the moment of observation, mmol/L	U	*p*
Patients with T2DM (*n* = 13)	10.4 ± 4.2	5.4 ± 0.7	5	$p \leq 0.01$
Patients with IGT (*n* = 5)	7.1 ± 1.5	4.7 ± 0.7	0.5	$p \leq 0.01$

Additional results of the study. There were no surgical, general or therapeutic complications. Mean bed-day of all patients was 4.4 ± 2.4 days.

Adverse events. No adverse events were noted.

In one case, a patient with T2DM underwent revision bariatric surgery – MGB-OAGB after sleeve gastrectomy due to obesity relapse. Before the first surgery, the patient's weight was 110 kg, BMI 36.8 kg/m^2; the patient took three OAD in combination with long-acting insulin (Levemir, 25 Uday). The level of fasting blood glucose was 12.3 mmol/L, HbA1c – 10.2 %, target HbA1c < 7 %. Preoperative preparation with short forms of insulin was performed in the hospital in order to normalize the blood glucose level. 10 months after sleeve gastrectomy the maximum %EWL is 78.8, %TWL is 24.5; the fasting blood glucose level is 6.0 mmol/L, HbA1c is 5.9 %. There was a withdrawal of one of the three OADs, the withdrawal of prolonged insulin. Target HbA1c values were noted within 6 months after surgery. Weight regain of up to 103 kg was recorded 47 months after sleeve gastrectomy, BMI – 34.4 kg /m^2, HbA1c – 6.3 %. A bariatric revision surgery was performed: sleeve gastrectomy in MGB-OAGB. At the moment of observation, the patient's weight was 90 kg, BMI – 30.1 kg/m^2, %EWL – 25.5, %TWL – 12.6 of the values before the second surgery; fasting blood glucose level – 5.9 mmol/L, HbA1c – 6.0 %.

DISCUSSION

This is a pilot study and is intended to assess the feasibility of conducting more solid studies in the future, which would include a larger number of patients, a longer follow-up period and a control group (patients with gastric bypass surgery).

Summary of the main result of the study. Sleeve gastrectomy demonstrates solid results in weight loss in obese patients. Moreover, the available metabolic effects of this operation allow to recommend it as the surgery of choice for patients with T2DM and IGT with a thorough clinical assessment of the patient.

CONCLUSION

The obtained results of the study indicate the undoubted advantage of sleeve gastrectomy both in terms of weight loss and compensation for type 2 diabetes mellitus and IGT. Improvement of the results of surgical treatment of this group of patients is possible by rational choice of the type of surgery. The performed study shows the expediency of further study of this problem and conducting more solid studies.

Conflict of interest

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

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ECONOMICS AND MANAGEMENT IN PUBLIC HEALTH SERVICE

ORGANIZATION OF WORK WITH UNDESIRED EVENTS WITHIN THE SYSTEM OF MEDICAL ACTIVITIES QUALITY AND SAFETY INTERNAL CONTROL WITH THE USE OF DIGITAL TECHNOLOGY

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ABSTRACT

In accordance with the current legislation a healthcare organization shall be obliged to provide conditions for safe delivery of healthcare to patients and medical personnel performance.
Implementation of effective model of medical activities quality and safety internal control producing meaningful result is a current license requirement to a healthcare organization.
Implementation of risk-oriented approach to medical activities quality and safety management is an important constituent of modern stage of the Russian Federation healthcare functioning.
It is necessary to understand sources of potential hazard within an organization, consider and analyze all undesired events and incidents arising in the process of a healthcare organization functioning and their reasons, take preventive actions to avoid them.
The tasks of digital transformation in the Russian Federation healthcare are development of integrated digital contour, transition to electronic document flow, reduction of medical personnel's time expenditure not involving delivery of healthcare.
Implementation of customer centricity and digitalization principles is an absolute trend of modern stage of development of the Russian Federation healthcare. It determines necessity of development of present-day accounting and analyzing data system on undesired events in a healthcare organization.
The article presents the experiment of Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul, Russian Federation) in establishing system of work with undesired events and managerial decision-making on their avoidance and prevention with the use of present-day digital technology resulting in credible frequency reduction of undesired events in the space of 2.5 years.

Key words: *undesired events in medical activities, medical activities quality and safety internal control, digital technology in healthcare*

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ОРГАНИЗАЦИЯ РАБОТЫ С НЕЖЕЛАТЕЛЬНЫМИ СОБЫТИЯМИ В СИСТЕМЕ ВНУТРЕННЕГО КОНТРОЛЯ КАЧЕСТВА И БЕЗОПАСНОСТИ МЕДИЦИНСКОЙ ДЕЯТЕЛЬНОСТИ С ПРИМЕНЕНИЕМ ЦИФРОВЫХ ТЕХНОЛОГИЙ

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РЕЗЮМЕ

Медицинские организации обязаны соблюдать условия для безопасного оказания медицинской помощи пациентам и работы сотрудников.
Применение эффективной модели внутреннего контроля качества и безопасности медицинской деятельности, дающей реальный результат, – это действующее лицензионное требование ко всем медицинским организациям.
Внедрение риск-ориентированного подхода к управлению качеством и безопасностью медицинской деятельности – важная составляющая современного этапа функционирования здравоохранения РФ.
Необходимо точно знать и идентифицировать источники потенциальной опасности внутри учреждения, учитывать и анализировать все нежелательные события и инциденты, которые возникают в процессе деятельности медицинской организации, причины и следствия, прогнозировать возможное наступление и своевременно принимать профилактические управленческие меры для их предотвращения.
Задачами цифровой трансформации в здравоохранении РФ являются создание единого цифрового контура, переход к электронному документообороту, сокращение временных затрат медицинских работников, не связанных непосредственно с оказанием медицинской помощи.
Внедрение принципов пациенто-центричности и цифровизации – абсолютный тренд современного этапа развития здравоохранения в РФ. Необходимость создания в медицинской организации современной системы учёта и анализа данных по нежелательным событиям направлена на реализацию трендов развития современной клиники и обеспечения безопасности пациентов и сотрудников.
В статье представлен опыт ФГБУ «Федеральный центр травматологии, ортопедии и эндопротезирования» Минздрава России (г. Барнаул) по созданию системы работы с нежелательными событиями и принятия управленческих решений по их устранению и предупреждению с применением современных цифровых технологий, что позволило получить достоверное снижение частоты нежелательных событий за 2,5 года.

Ключевые слова: *нежелательные события в медицинской деятельности, внутренний контроль качества и безопасности медицинской деятельности, цифровые технологии в здравоохранении*

Для цитирования: Колядо Е.В., Пелеганчук В.А., Шульц Т.Е., Повалихин А.Н., Лазарева В.В. Организация работы с нежелательными событиями в системе внутреннего контроля качества и безопасности медицинской деятельности с применением цифровых технологий. *Acta biomedica scientifica*. 2023; 8(1): 218-227. doi: 10.29413/ABS.2023-8.1.22

INTRODUCTION

Ensuring the quality and safety of medical activity remains one of the topical issues of domestic healthcare in current conditions, despite the research conducted in this area and the numerous approaches proposed to solve this problem [1].

At the modern stage of the development of healthcare in the Russian Federation, the requirements for the quality of medical care and the safety of medical activities have been strengthened, the requirements for the internal control system have been formed, which is implemented in the latest changes in legal regulation [2]. Implementation of effective model of medical activities quality and safety internal control producing meaningful result is a current license requirement to a healthcare organization [3].

Medical activities quality and safety internal control is carried out in order to ensure the rights of citizens to receive medical care of the necessary volume and proper quality in accordance with current legislation and compliance with mandatory requirements for ensuring the quality and safety of medical activities [1].

To achieve this goal, a healthcare organization needs to create an effective system of medical activities quality and safety internal control based on current regulatory requirements and modern technology, including digital technology.

The results of primary audits of the quality and safety of medical activities of 30 healthcare organizations in 11 regions of the Russian Federation by multidisciplinary working groups of experts revealed the existing systemic problems in the organization of work and the lack of unified approaches in almost all sections of this area in general and in the organization of recording and analysis of adverse events in particular [4].

Medical activity belongs to the category of very high-risk industries. Risk management helps to prevent, minimize or eliminate possible harm to the life and health of patients and personnel. The introduction of a risk-based approach to managing the quality and safety of medical activities is an important component of the current stage of the functioning of healthcare in Russia. Risk management is an integral part of the organization's management and is of fundamental importance [1, 5, 6].

Review data from different authors recorded the proportion of identified adverse events in the implementation of medical activities from 2.9 to 16.6 % of all hospital admissions in an analysis of up to 30,000 in-patient medical records [7].

The systems of organization of reporting on adverse events and errors in the field of healthcare, functioning in many foreign countries, differ in their organizational structure (public, private, public – agencies, foundations, relevant ministries and committees to which all healthcare organizations of the country are required to send their information), but almost everywhere they have a national or governmental status. Most countries have legislation providing for the confidentiality of information contained in reports. However, sociological surveys have shown that about 70 % of the population would like to have free access to information about adverse events in the healthcare area and healthcare organizations [8].

In the Russian Federation Presidential Decree No. 474 dated July 21, 2020 "On the national development goals of the Russian Federation for the period up to 2030", two of the five national development goals of the Russian Federation are devoted to the preservation of public health and digital transformation. The basic principle of management in all sectors of the state is the orientation towards social results for people, the achievement of indicators of national development goals. Result-oriented performance also implies the creation of risk management system – identifying risks in a timely manner and establishing actions to prevent and mitigate them.

Implementation of customer centricity and digitalization principles – improving the quality of life and the level of public confidence, changing approaches to working with people to solve their life situations, proactive informing about new opportunities, including through the introduction of new digital solutions – is an absolute trend of the modern stage of development of various industries in the Russian Federation [9, 10, 11].

Citizens' demands for the high quality of public services, in general, for the quality of life – housing, medicine, education – are constantly increasing, and the growth of the population's requests is outpacing the rate of the changes [9]. The protection of patients' rights, the activities of human rights organizations have acquired a separate focus and degree of tension. Digital technologies create new opportunities. In addition, digitalization increases the availability of services [9].

The strategic goal in the development of modern medicine is to create an integrated digital contour based on Uniform State Health Information System (USHIS) and to manage the indicators of achievement in an incident-management mode [12, 13].

The main tasks of digital transformation are the transition to electronic document flow in the Russian Federation healthcare; reduction of medical personnel's time expenditure not involving delivery of healthcare. At the same time, the problems of the current state of healthcare that can be solved with digitalization are the following: weak data management due to the lack of integrated applications, unified reference and regulatory information management environment; increased workload on healthcare workers as a result of working with multiple systems and a large amount of manual data input, the need to maintain documentation, including medical, in paper form; long terms, difficulties in the development and implementation of "end-to-end" services and business processes due to the need to integrate several information systems, registries and registers; fragmentation of healthcare information systems, lack of uniform standards of information interaction; limited interdepartmental electronic interaction [12].

In 2022, the Accounts Chamber of the Russian Federation analyzed the current state of healthcare informational support and identified a number of problems that hin-

der the digital transformation of this sphere [14]. The quality of data is negatively affected by the need to multiple information input. According to the inspection, medical workers have to input patient data into several unrelated information systems at the same time. Insufficient formalization of the processes carried out in healthcare organizations significantly hinders their automation and digital transformation, and also leads to different approaches of regions and healthcare organizations to the implementation of the functionality of health information systems. Information technologies as a tool, in addition to solving control and recording tasks, must ensure a reduction in the labor costs of healthcare workers. The introduction of information systems without rejection of hard-copy document flow significantly reduces the productivity of medical personnel and creates an additional workload for doctors. Almost 90 % of doctors interviewed for the inspection believe that digitalization is necessary. However, only 30 % of healthcare workers noted the effect of informational support, since it has become easier to process patient documentation. 27 % noted a reduction in time spent on work [14].

Digital system architecture of the Ministry of Health of Russia, The Federal Fund for Mandatory Medical Insurance and other authorities, as well as the requirements for regional systems must ensure seamless integration and the creation of a single information space in healthcare. The key issue of unification of business processes on the basis of unified directories, data models and registers must be addressed as quickly as possible.

THE PROCEDURE FOR RECORDING AND ANALYSIS OF ADVERSE EVENTS AS A PART OF INTERNAL QUALITY CONTROL AND SAFETY OF MEDICAL ACTIVITIES

The main objectives of Internal Quality Control and Safety of Medical Activities are to improve approaches to the implementation of medical activities to prevent, identify and prevent risks to the life and health of citizens and minimize the consequences of their occurrence, prevent violations in the provision of medical care and make management decisions to improve approaches to the implementation of medical activities [2].

One of the actions carried out as a part of Internal Quality Control and Safety of Medical Activities is the recording of adverse events during the implementation of medical activities (facts and circumstances that pose a threat of causing or entailing harm to the life and health of citizens and/or healthcare workers, as well as leading to an extension of the terms of medical care) [2, 15].

Many processes in the field of healthcare do not have legal regulation, and their implementation differs between healthcare organizations and regions, which complicates their further automation through information systems. This leads to a different approach in the implementation of the functional modules of state and health information systems in the subjects of the Russian Federation, in healthcare organizations and complicates the "end-to-end" exchange of information.

The procedure for recording and analysis of adverse events in the implementation of medical activities is not regulated by federal legislation as a whole (the procedure for reporting, the procedure for recording and analysis, consideration, etc.). The regulation of this issue is in international standards, which causes certain difficulties, different approaches are used in organizations when implementing these actions [16].

There is no exhaustive universal classifier of adverse events in the implementation of medical activities, which healthcare organizations could use as a basis for the organization of internal recording and analysis of indicators, the formation of unified reporting, adapting it to the specifics of their organization.

There is a regulation on certain thematic areas, which is not sufficiently harmonized between them for the purposes of the process approach: Order of Roszdravnadzor (Federal Service for Supervision in Healthcare) No. 4513 dated May 20, 2021 approved the classification of adverse events connected with medical devices circulation; Order of the Ministry of Health of the Russian Federation No. 1108n dated November 29, 2021 approved the procedure for revealing and recording at a healthcare organization of cases of infectious diseases connected with delivery of healthcare, nomenclature of infectious diseases connected with delivery of healthcare subject to revealing and recording at a healthcare organization; Order of Roszdravnadzor (Federal Service for Supervision in Healthcare) No. 1071 dated February 15, 2017 approved the procedure for pharmacovigilance; Order of the Ministry of Health of the Russian Federation No. 1113n dated October 19, 2020 approved the procedure for reporting by subjects of medical devices circulation of facts and circumstances creating danger to life and health of general public and medical personnel during use and operation of medical devices. However, the spectrum and list of undesired events are much broader, which requires systematization at the federal level [17–20].

Global trends in the digital transformation of the healthcare industry and priority areas for the development of this type of technology allow electronic recording and analysis systems to be classified as Predictive Analytics – the intelligent use of data, predictive modeling of future events, support and justification of managerial decision-making [21].

According to experts, Health Information Technology (HIT) is the most effective tool for improving the quality, efficiency, safety of medical care, but it is also the most expensive. Studies show that in institutions that have switched to an electronic incident reporting system (web systems), the frequency and timeliness of reporting, the accuracy of key indicators and the systematic organization of work in this area have increased [22].

An important issue, which also represents a traditional problem, is the system of electronic intradepartmental and interdepartmental document flow for the operational formation and provision of various forms of reporting to dif-

ferent departments and divisions, which, according to current legislation, is to be put into operation in the Russian Federation on December 31, 2024 [23].

The above arguments became the basis for setting the goal of creating a system of recording and analysis of adverse events (AEs) in our healthcare organization for managerial decision-making on avoidance and prevention of risks with the use of digital technology, reducing of adverse events. Creation of a unified digital system in the Russian Federation seems to be the optimal solution to this issue.

To achieve this goal, the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul) has developed a "Procedure for recording adverse events in the implementation of medical activities and other incidents", containing the following sections:

- goals, objectives, principles of operation, classification and basic definitions, an approximate list of AEs in the implementation of medical activities and other incidents (including markers (signs) of adverse events);
- the procedure for reporting (registration) by the employees of the institution, patients and visitors about an adverse event;
- unified form of AE recording;
- the procedure of AE investigating, identifying the type of AE by consequences;
- the procedure for calculating and analyzing parameters, monitoring the dynamics and trends of AE for established periods;
- the form of the corrective action plan, the procedure for monitoring the effectiveness of the actions taken;
- informing employees, the procedure for internal personnel training, the procedure for feedback.

Electronic Adverse Event Recording System (Incident notifications) is a web-based system that allows employees of a healthcare organization and patients to voluntarily report problems that have occurred.

The system is integrated with the internal Medical Information System (MIS) and the Electronic Health Record (EHR) of a patient for automatic (without personnel involvement) detection, reporting and recording of information about an adverse event with the help of key trigger indicators (critical values of laboratory and other instrumental indicators, the volume of blood loss, labeled medical formulations recorded in the medical documentation, other).

The developed electronic adverse event recording system has a number of advantages:

- immediately, in real time, automatically detects, independently detects and reports serious incidents to the medical chat of the institution (excludes the presence of "unrecorded" incidents);
- automates and simplifies data input and analysis, reduces the work time with this incident on its processing (ready-made templates have been created) and informing all officials (operational information about the adverse event is immediately reported to the hospital chat);
- minimizes the involvement of personnel in detection and recording of the adverse event;
- standardizes the procedure and structure of reporting, the process of analyzing the causes of the adverse event and the development of corrective actions;
- helps to improve clinical processes (based on the developed SOP and algorithms);
- helps to predict and identify potential risks.

THE SYSTEM OF DEALING WITH ADVERSE EVENTS

The Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul) has a system for dealing with adverse events, structured by stages (Fig. 1).

A computer program for recording adverse events has been created on the Bitrix platform (contains 66 accounting parameters), which is implemented on the website of the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul).

The first stage is the establishment of the fact of an adverse event, notification (registration) of the AE in the electronic recording system (computer program): employees (medical and non-medical personnel, patients and visitors), MIS (according to established triggers: words and critical levels of monitoring indicators), the initial processing of the notification by the Department of Internal Quality Control and Safety of Medical Activities.

The second stage is the analysis of the AE by consequences (errors with little or no harm, errors with significant harm and extreme events that are considered based on significance within 48 hours (urgent) or monthly).

The third stage is an internal inspection of the AE by a task force using the RCA (root cause analysis) method, preparation of a plan and implementation of corrective actions, consideration by a medical commission (efficiency control), changes in internal algorithms (orders).

The fourth stage is the analysis of monitoring indicators for the established periods, identification of dynamics, informing employees, additional internal personnel training, feedback from patients and visitors (if necessary).

RESULTS OF FUNCTIONING OF THE SYSTEM OF HANDLING WITH ADVERSE EVENTS AS A PART OF INTERNAL QUALITY CONTROL AND SAFETY OF MEDICAL ACTIVITIES

The system organizational actions carried out as a part of Internal Quality Control and Safety of Medical Activities, based on strict recording of AE with the use of digital technologies, root cause analysis of occurrence and development of a set of preventive actions, allowed to reduce the values of the main control monitoring parameters of AE at the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul) for 2.5 years (2020–2022); thus, we have implemented actions to improve the level of safety for patients and employees.

Over 2.5 years (2020–2022) the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Bar-

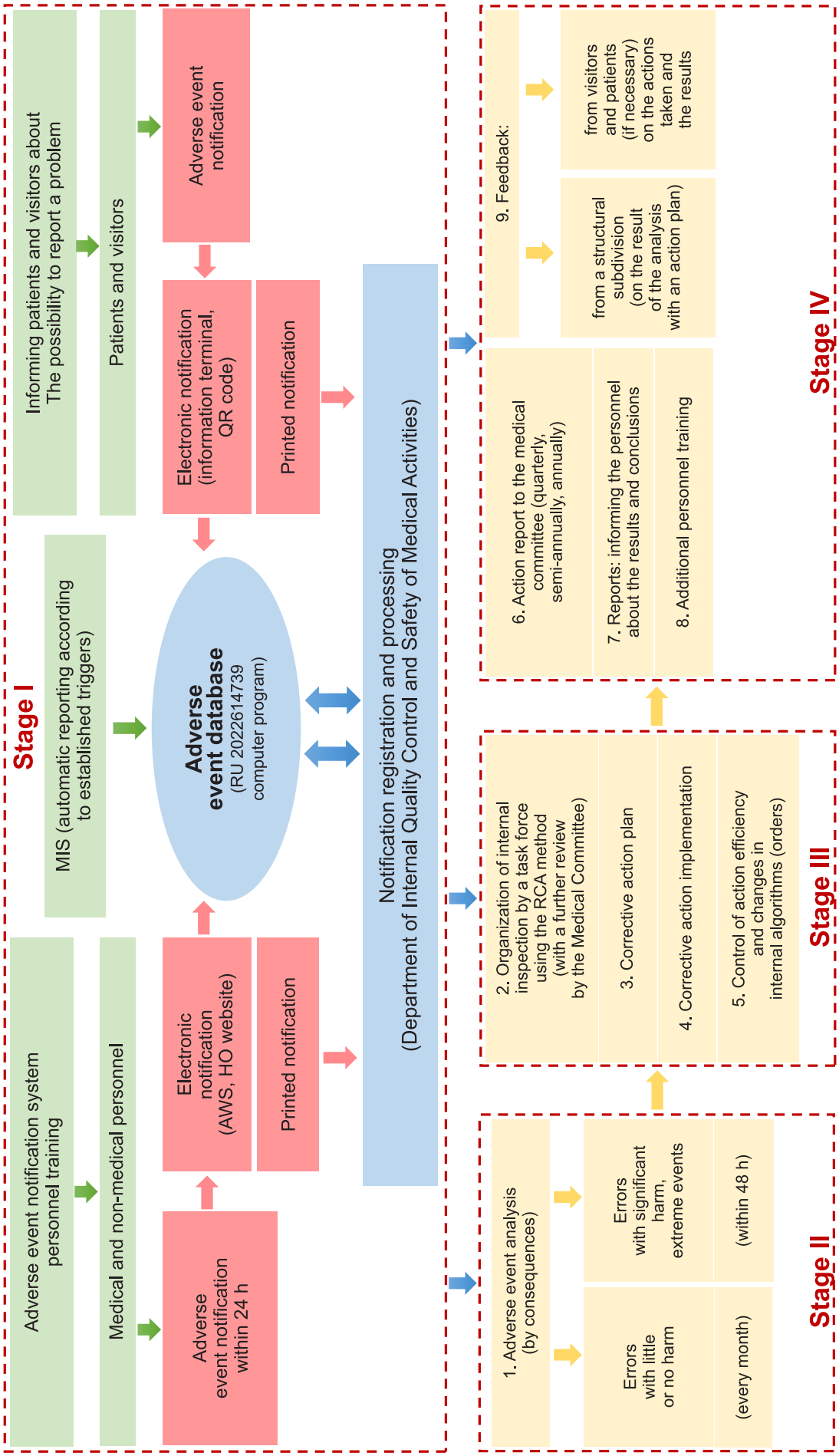

FIG. 1.
Stages of work with undesired events at the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul)

naul) has analyzed 19,639 cases of hospital admission to the "Traumatology and Orthopaedics" and "Neurosurgery" departments and registered 269 adverse events.

On March 24, 2022, we successfully completed the procedure of state registration of the computer program "Recording of adverse events during implementation of medical activities" in the Federal Service for Intellectual Property (Rospatent), which complies with the requirements of legislation on information security and personal data protection. The Certificate of State Registration (No. 2022614739) was received [24].

The indicators of the AE rate (per 1000 treated patients) were established in the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul) according to our approved internal order rubrics from the highest to the lowest: 1st – the AE rate parameter, events during the treatment of the patient – 2.85, 2nd – other incidents (non-medical) – 2.24, 3rd place – events related to surgical interventions or other procedures – 1.22, 4th – markers (signs) of adverse events during the implementation of medical activities – 1.02, 5th – events related to the use of medical devices – 0.61, 6th – events related to the use of medicines – 0.41, 7th – events related to infection and events related to the implementation of anesthesia, – 0.2 for each category.

We rank all structural divisions of the Federal Center for Traumatology, Orthopedics and Endoprosthetics (Barnaul) according to the final indicators of the rate of adverse events from the highest parameter to the lowest. A rating of departments is formed according to the AE indicators, which is used in the system of remuneration and bonuses of employees as one of the supporting stimulating indicators established by a local legal act.

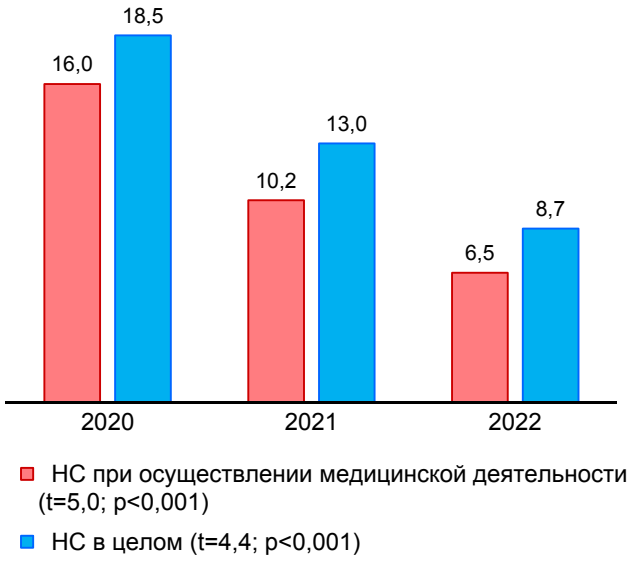

FIG. 2.
Trend data of undesired events frequency in the course of medical activities and other incidents in the years 2020–2022 (for 1000 treated patients)

The introduction of the system for managing adverse events as a part of Internal Quality Control and Safety of Medical Activities, including streamlined procedures for registration, analysis, control and managerial decision-making aimed at prevention and improvement, allowed us to obtain a significant reduction in the rate of adverse events in a healthcare organization for 2.5 years (Fig. 2).

We have registered a decrease in the AE rate in the implementation of medical activities (per 1000 treated patients) from 16.0 in 2020 to 10.2 in 2021 and 6.6 in the first half of 2022 ($p = 0.0001$; $p < 0.001$); decrease in the AE rate (in general, including non-medical, other) – from 18.5 in 2020 to 13.0 in 2021 and 8.7 in the first half of 2022. ($p = 0.0003$; $p < 0.001$).

Thus, a systematic approach to the identification, recording, analysis of adverse events using digital technologies, operational management actions as a part of Internal Quality Control and Safety of Medical Activities allowed to successfully achieve reducing the rate of adverse events in the implementation of medical activities for 2.5 years (2020–2022).

Conflict of interest

There are no obvious and potential conflicts of interest related to the publication of this article.

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EXPERIMENTAL RESEARCHES

PATHOLOGICAL CHANGES OF THE SPLEEN IN MICE INFECTED WITH INFLUENZA AGAINST THE BACKGROUND OF THE USE OF SAPONIN TAUROSIDE Sx1

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ABSTRACT

Background. *It is well known that viral infections are able to cause an imbalance of the interferon system and inhibition of cellular and phagocytic reactions of the body. One of the possible solutions of the flu treatment problem may be the application of immunomodulators of native plant origin since the influenza virus possesses a suppressive effect on cellular immunity and the interferon system.*

The aim. *To evaluate the effect of saponin tauroside Sx1 obtained from Crimean ivy leaves on histological changes in the spleen of mice infected with influenza A/WSN/1/33(H1N1) virus.*

Material and methods. *We used 78 male BALB/c mice weighing 16–18 g which were divided into the groups: control (K; n = 12); healthy animals treated with saponin (KS; n = 22); animals infected with influenza virus A/WSN/1/33(H1N1) (V; n = 22); infected animals treated with saponin tauroside Sx1 twice a day for 3 days (SV; n = 22). Histological studies of the spleen were performed on the days 4 (subgroups V, SV, KS) and 14 (2V, 2SV, 2KS).*

Results. *The spleen tissue of the KS subgroup demonstrated hyperplasia of the white pulp in the form of lymphoid nodules expansion. On the days 4 in the KS subgroup a statistically significant increase in the total area of the lymphoid nodules by 3.9 times compared to the K subgroup was observed. In subgroup V, there was a sharp decrease in the area of white pulp. In subgroup 2V, areas of lymphoid nodules were almost indistinguishable. Applied correction in the SV and 2SV subgroups significantly ceased the damaging effect of the virus: the lymphoid nodules area increased by 2.7 times in the 2SV subgroup compared to 2V.*

Conclusion. *Infection with H1N1 influenza virus leads to a compensatory activation of the immune response, however, on the day 14 a pronounced depletion of the white pulp of the spleen is observed. The introduction of saponin tauroside Sx1 enhanced the functional activity of the spleen due to an increase of the white pulp area.*

Key words: *triterpenoid saponin, influenza virus, spleen, immunomodulation*

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ПАТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ СЕЛЕЗЁНКИ У МЫШЕЙ, ЗАРАЖЁННЫХ ГРИППОМ, НА ФОНЕ ПРИМЕНЕНИЯ САПОНИНА ТАУРОЗИДА Sx1

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РЕЗЮМЕ

Обоснование. *Известно, что вирусные инфекции вызывают дисбаланс системы интерферонов, угнетение клеточных и фагоцитарных реакций организма. Одним из возможных решений проблемы лечения гриппа может явиться применение отечественных иммуномодуляторов растительного происхождения, поскольку вирусы гриппа оказывают супрессивное действие на клеточный иммунитет и систему интерферонов.*

Цель исследования. *Оценить влияние перорального введения сапонина таурозида Sx1, полученного из листьев крымского плюща, на гистологические изменения селезёнки мышей, заражённых вирусом гриппа A/WSN/1/33(H1N1).*

Материал и методы. *Использовали 78 самцов мышей линии BALB/c весом 16–18 г, разделённых на группы: контрольная, здоровые животные (K; n = 12); контрольная, здоровые животные, получавшие сапонин (KS; n = 22); животные, зараженные вирусом гриппа A/WSN/1/33(H1N1) (V; n = 22); животные, зараженные вирусом гриппа A/WSN/1/33(H1N1) и получавшие сапонин таурозид Sx1 дважды в день в течение 3 дней после заражения (SV; n = 22). Гистологические исследования селезёнки проводили на 4-й (подгруппы V, SV, KS) и 14-й день (2V, 2SV, 2KS).*

Результаты. *В ткани селезёнки подгруппы KS отмечалась выраженная гиперплазия белой пульпы в виде расширения лимфоидных узелков. На 4-й день в подгруппе KS наблюдалось статистически значимое увеличение общей площади лимфоидных узелков по сравнению с подгруппой K в 3,9 раза. В подгруппе V отмечалось резкое уменьшение площади белой пульпы. В подгруппе 2V зоны лимфоидных узелков были практически неразличимы. На фоне коррекции в подгруппах SV и 2SV повреждающее воздействие вируса было выражено значительно меньше: площадь лимфоидных узелков увеличивалась в 2,7 раза в подгруппе 2SV по сравнению с 2V.*

Заключение. *Инфицирование вирусом гриппа H1N1 приводит к компенсаторной активации иммунного ответа, однако на 14-е сутки наблюдается выраженное истощение белой пульпы селезёнки. Введение сапонина таурозида Sx1 положительно влияет на функциональную активность селезёнки за счёт прироста площади белой пульпы.*

Ключевые слова: *тритерпеновый сапонин, вирус гриппа, селезёнка, иммуномодуляция*

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INTRODUCTION

Influenza, like other acute respiratory viral infections (ARVI), undoubtedly occupies a leading place in the structure of respiratory infectious diseases. According to the World Health Organization (WHO), up to 646,000 patients die from seasonal influenza worldwide every year [1].

During the pandemic of a new coronavirus infection, the danger of influenza should not be underestimated. Influenza remains one of the most important health problems and poses a serious threat to adults and children in all countries [2]. Assessments indicate that annual influenza can affect 5–20 % of adults and 20–30 % of children, and when pandemics occur the incidence of influenza can rise up to 50 % [2]. Influenza complications are also known to be at high risk among young children during the first years of life, adults aged 65 years and over, pregnant women and people with chronic medical conditions, which is due to the immunosuppressive effect of the influenza virus, which aggravates the severity of the existing chronic somatic nosology [2]. Every year the influenza virus mutates, becoming resistant to many antiviral drugs, which also leads to severe complications and lethal outcomes. Chronic cardiovascular or pulmonary disease, obesity, pregnant women and tobacco smokers have been shown to increase the risk of being fatally ill with influenza tenfold [3–5].

Etiotropic anti-influenza drugs are the basis of antiviral chemotherapy, but their number is very limited. According to the clinical guidelines of the Ministry of Health of Russia "Influenza among adults", four drugs are distinguished as antiviral drugs with direct antiviral effect: oseltamivir, zanamivir, riamilovir and umifenovir [6]. Considering the widespread resistance of current strains to adamantane-based drugs and the high likelihood of resistance to neuraminidase inhibitors, as well as the severe side-effects of commonly used antiviral drugs, the search for effective and safe immunostimulants and antivirals to prevent and treat influenza is vital. Such drugs should not be significantly affected by viral variability and should have universal antiviral properties. After the acute and viraemia have subsided among severe influenza patients, the use of immunomodulators, including herbal supplements, to stimulate immune activation and seroconversion is recommended [7, 8].

Saponins make up an extensive group of plant glycosides that inhibit the development of fungi, bacteria, viruses and protozoa, stimulate humoral and cell-mediated immunity, and are also able to enhance the immune response during vaccination [9–11]. When studying the properties of saponins isolated from Crimean ivy *Hedera taurica* (Hibberd) Carrière, it was proved that tauroside H_2, -St-K and -I can enhance antibody synthesis in mice, and tauroside Sx1 is able to inhibit the growth of fungi of the genus *Candida* and enhance the resistance of mice to fungal infection [12, 13]. Oral administration of 200 μg of tauroside Sx1 to mice infected with the influenza virus has been proven to cause a 1.5-fold increase in their survival compared to the survival of infected mice in the control [14]. At the same time, the tissue, cellular and molecular mechanisms of the development of the immune response induced by saponins, as well as their effect on the immunogenesis organs, remain practically unstudied.

The spleen is a highly organised and the largest polyfunctional peripheral organ of the immune system, in which the intensity of immune and filtration processes closely correlates with the architectonics of its white and red pulps as well as the quantitative ratio of the areas occupied functionally by the different segments with their cellular composition. At the same time, the spleen shows a high degree of reactivity with cytological restructuring of its immunocompetent structures under the influence of various endogenous and exogenous factors, including the background of infection. The absence of the spleen is known to cause increased susceptibility to systemic bacterial infections, while the role of the spleen in antiviral immunity is less well studied [15].

Data from other researchers indicate that the rat spleen is able to respond to the introduction of immunomodulators with pronounced morphological changes, which, as a rule, is accompanied by hyperplasia of B-dependent zones [16]. Thus, the choice of the spleen as an experimental model for evaluating the effectiveness of immunomodulatory effects is justified by the fact that it participates in almost all immune and hematopoietic processes, being the center of antigen-dependent proliferation and differentiation of components of both cellular and humoral immune response, its activation, as well as the production and secretion of specific immunoglobulins.

THE AIM OF THE STUDY

To evaluate the effect of oral administration of saponin tauroside Sx1, obtained from Crimean ivy leaves, on histological changes in the spleen of mice infected with influenza A/WSN/1/33(H1N1) virus at different periods of influenza infection.

MATERIALS AND METHODS

Influenza virus (IV) A/WSN/1/33(H1N1) adapted to mice was used in this study. The initial strain of IV was obtained from the collection of the D.I. Ivanovsky Research Institute of Virology of the Russian Academy of Medical Sciences (Moscow). The adaptation of IV to mice and the production of a lethal strain were carried out with repeated passaging of the virus through the lungs of the animals [17]. The initial virus-containing liquid was injected into mice intranasally under light ether anesthesia. After 3 days, the lungs were extracted from the animals and lung tissue homogenate was obtained under sterile conditions, after centrifugation, the supernatant fluid was injected intranasally into the mice. The procedure was repeated three times, the last lung homogenate was injected into 10-day-old chicken embryos in order to accumulate the virus. After six series of similar passages made on mice and chicken embryos, the resulting allantois fluid contained an influenza virus that was lethal to mice. The virus content in the samples was tested

by haemagglutination reaction with chicken erythrocytes [17]. The adaptation of the virus to reproduce in the lungs of mice resulted in a lethal influenza model, which was subsequently used in our studies. In hemagglutinin yield reduction assay of chicken erythrocytes, the dynamics of accumulation of antihemagglutinin antibody titers in the sera of infected animals on the days 4, 7, 14 and 18 after infection was determined. The dynamics of influenza virus accumulation in the lungs of mice on days 2–6 after infection was also studied. An anti-haemagglutinin antibody titer was found to be 80 ± 15.1 on day 4 post-IV infection. The infectious titer of the virus in the lungs of mice at the same time of infection was 2.2×10^3. By the day 14 of the experiment, the antibody titer increased almost 4 times and amounted to 213.3 ± 53.3. The average lifespan of mice during experiments ranged from 9.9 ± 1.3 to 11.5 ± 2.1 days, depending on the weight and age of the animals [14].

In the experiment, 78 male BALB/c mice without external pathological signs, weighing 16–18 g and 4–6 weeks of age were used. They were divided into the following groups and subgroups:

1. Healthy animals as a control group: subgroup K receiving 50 µl of saline orally during 3 days, withdrawn from the experiment on day 4 (n = 6), and subgroup 2K receiving saline orally following the same regimen, withdrawn from the experiment on day 14 (n = 6).

2. Healthy animals receiving oral saponin for 3 days at a concentration of 5 mg/ml (dose 200 µg/mouse/day): subgroup KS, withdrawn from the experiment on day 4 (n = 11), and subgroup 2KS, receiving saponin according to the same regimen, withdrawn from the experiment on day 14 (n = 11).

3. Animals infected intranasally with influenza A/WSN/1/33(H1N1) virus without correction: subgroup V, withdrawn from the experiment on day 4 (n = 11), and subgroup 2V, withdrawn from the experiment on day 14 (n = 11).

4. Animals infected intranasally with influenza A/WSN/1/33(H1N1) virus and receiving oral saponin at a concentration of 5 mg/ml (dose 200 µg/mouse/day): subgroup SV, derived from the experiment on day 4 (n = 11), and subgroup 2SV, receiving saponin according to a similar regimen and withdrawn from the experiment on day 14 (n = 11).

Study design

Male mice weighing 16–18 g were infected intranasally with influenza A/WSN/1/33(H1N1) virus under brief ether anesthesia by injecting 50 µl of allantois fluid containing 5 LD_{50} of the virus. Aliquots of a single pool of allantois fluid were used, frozen and stored at a temperature of –20 °C. Clinical symptoms confirming the development of viral infection were progressive weight loss and the development of respiratory failure (rapid breathing over 200 respiratory movements per minute with involvement of the ancillary muscles, cyanosis of the tail, ears and limbs). On the days 4 and 14 after infection, the animals were withdrawn from the experiment by decapitation using ether anesthesia, then the spleen was removed for further follow-up study.

A therapeutic regimen of saponin administration was used, which has previously been shown to be effective in animals during experimental influenza infection and influenza vaccination [13, 14]. A triterpene glycoside with the formula 3-O-a-L rhamnopyranosyl (1→2)-a-L-arabinopyranoside hederagenin, abbreviated as saponin tauroside Sx1, isolated at the Department of Physical and Analytical Chemistry of the Vernadsky Taurida National University from the Crimean ivy *H. taurica* by Professor V.I. Grishkovets [18, 19], was used in the experiment.

Histological study methods

Spleen tissue was fixed in 10% neutral buffered formalin with subsequent treatment in alcohol with increasing concentration, embedding in paraffin and preparation of 4 µm-thick sections according to generally accepted histological methods [20]. Paraffin sections were stained with hematoxylin and eosin to carry out a qualitative assessment of morphological transformations followed by morphometric assessment by tracing the contours of scans of longitudinal and anatomically whole histological sections of the spleen obtained on a Leica scanner with a light pen in Aperio Image Scope morphometric and structural image analysis software (Leica Biosystems, USA). The total area (S, mm^2) and the percentage ratio (relative area) of red and white pulp, as well as the area of lymphoid nodules were determined on the sections. The measurements were taken at a magnification of 200×.

All histological studies were carried out in the Center for Collective Use "Molecular Biology" on the basis of the Central Research Laboratory of the Institute of S.I. Georgievsky Medical Academy of the V.I. Vernadsky Crimean Federal University. The research was carried out with the financial support of the Ministry of Science and Higher Education of the Russian Federation, Priority-2030 program No. 075-15-2021-1323.

Ethical review

The study was approved by the Ethics Committee of the V.I. Vernadsky Crimean Federal University (Protocol No. 10 dated November 23, 2021). The animals were kept in the vivarium on a standard diet with free access to food and water under natural light conditions and care was provided according to the requirements and rules governing the treatment of laboratory animals in accordance with the "Rules for carrying out work using experimental animals" (Order of the Ministry of Higher and Secondary Education No. 724 dated Noovember 13, 1984).

During the study, the principles of the Helsinki Declaration adopted by the General Assembly of the World Medical Association (2000) were observed. The animals were withdrawn from the experiment in accordance with the "International Recommendations (Code of Ethics) for conducting Biomedical Research Involving Animals" of the Council for International Organizations of Medical Sciences (CIOMS) (1985) and the rules of laboratory practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267 dated June 19, 2003).

Statistical data analysis

Histomorphometric data were processed statistically using the Statistica 10.0 software (StatSoft Inc., USA). The mean

(M), the standard error of the mean (m) and the standard deviation (σ) of the areas of red and white pulp, as well as lymphoid nodules were calculated.

During statistical data processing, the variation series were checked for the normality of the distribution according to the Shapiro – Wilk test between the control and experimental groups of laboratory animals. A Student's t-test confidence interval was used to assess differences in qualitative characters. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Most of the spleen parenchyma of control mice was represented by a red pulp consisting of venous sinuses and a net of reticular strands, in the loops of which there are blood cells counts. The white pulp was defined as lymphoid nodules and periarterial lymphoid sheaths with pronounced zonal distribution. Small and medium-sized lymphocytes, plasma cells, reticular cells and macrophages were visualised in the periarteriolar lymphocyte sheaths. In the central part, located directly near the arterial wall, the dominant cell population was specific macrophages.

The following zones were identified in the lymph nodes: periarterial, mantle, marginal and germinal center. The eccentrically located periarterial zone was represented by a pool of mature lymphocytes that surround the central arteriole. A germinal centre with lymphoblasts was visualised adjacent to the periarterial zone. The above-described zones were surrounded by a mantle zone, represented mainly by macrophages and in smaller numbers by erythrocytes, and small lymphocytes, and tissue basophils. The marginal zone was separated from the mantle marginal sinus, localised around the periphery of the lymph node and consisted of medium-sized lymphocytes and macrophages (Fig. 1a).

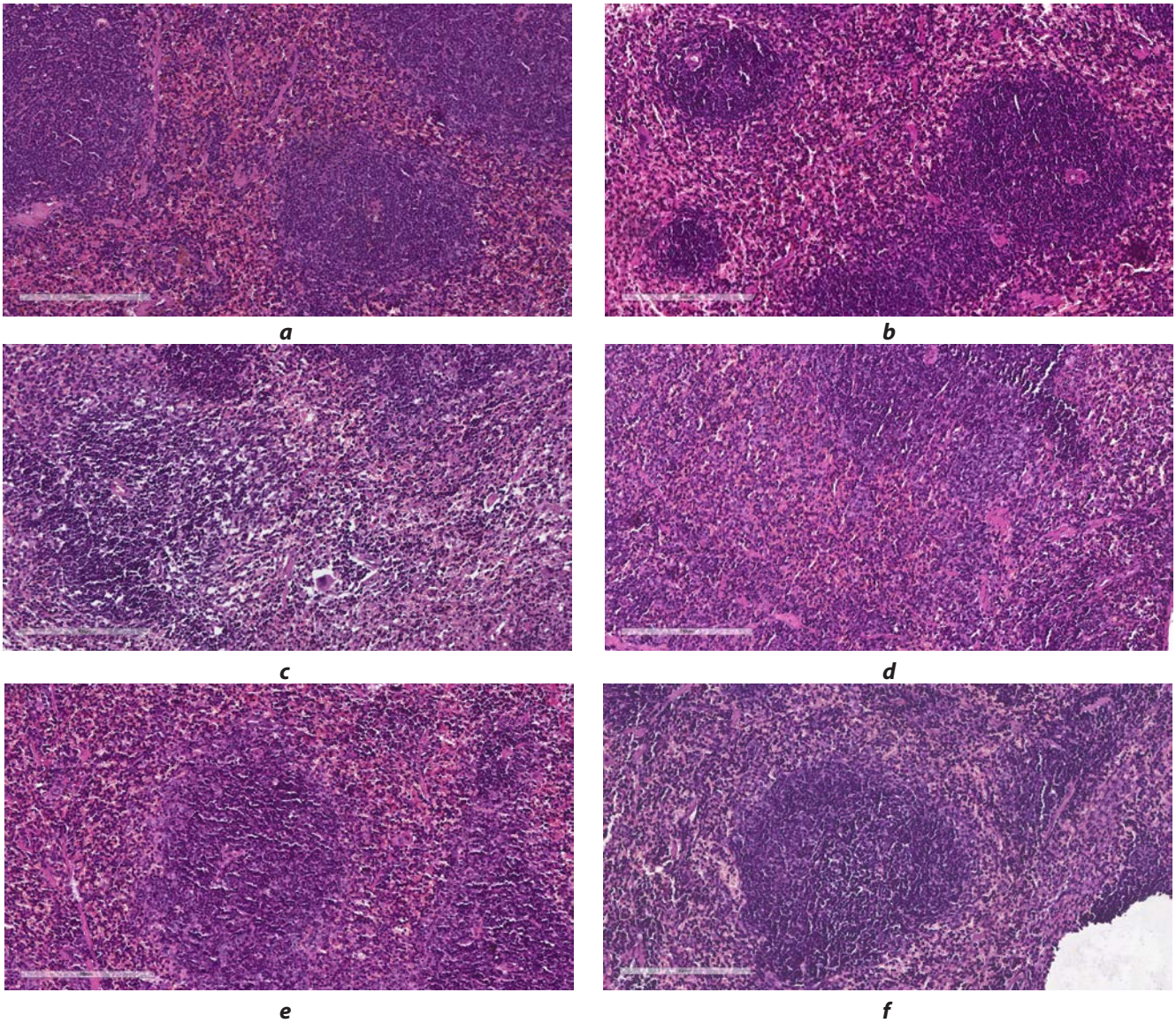

FIG. 1.

*Histological changes in the spleen of BALB/c mice: **a** – control group; **b** – subgroup KS; **c** – subgroup V; **d** – subgroup 2V; **e** – subgroup SV; **f** – subgroup 2SV. Hematoxylin-eosin stain, magnification ×200 Haematoxylin-eosin staining, magn. 200x*

When the histological structure of the spleen tissue was analyzed, the most significant intergroup differences were detected in the K and KS subgroups. In the spleen tissue of the KS subgroup mice a significant hyperplasia of the white pulp in the form of enlarged lymphoid nodules with an increase in the germinal center was observed. Lymphoid nodules occasionally merged with each other and had well-defined zones (Fig. 1b).

Exposure to influenza virus has resulted in significant changes in subgroups V and 2V. In the spleen of mice from subgroup V, there was an increase in the megalokaryocytic reaction, "blurring" of zones and erasing the boundaries of nodules, as well as a decrease in the area occupied by the white pulp. The visible "overgrowth" of the lymphoid nodules was often caused by the fusion of the nearest nodules, which led to a decrease in their total number (Fig. 1b). In subgroup 2V, the histological zones of lymphoid nodules were almost indistinguishable and often there was a complete disappearance of germinal centers, which could indirectly indicate inhibition of lymphocytic proliferation processes (Fig. 1d). Dilated sinusoids often showed hemostasis, neutrophil accumulation, as well as hyperplasia of macrophage cells.

Against the background of drug therapy in the SV and 2SV subgroups, the negative effect of the virus on the spleen of mice was significantly less pronounced. The following histological changes in these subgroups should be noted: despite a significant number of megakaryocytes, a sufficient number of lymphoid nodules, both with and without light germinal centers, were detected in the spleen tissue. Periarterial lymphoid sheaths were visualized, in some of which zoning remained preserved (Fig. 1d, e).

According to morphometric analysis, on the day 4 after saponin administration, as well as against the background of viral infection, a statistically significant increase in the average lymphoid nodule area was observed in all experimental subgroups compared to the control (Table 1). A pronounced statistically significant increase in the mean lymphoid nodules area by a factor of 3.4 has been observed in the healthy animals treated with saponin (KS) compared to the control subgroup (K). In the subgroups of mice V and SV infected with the influenza virus, the increase in the average area of lymphoid nodules was 2.3 and 2.1 times, respectively.

On the 14th day of the experiment, a statistically significant difference in the average lymphoid nodules area between the two subgroups 2K and 2KS was retained, whereby saponin administration more than doubled the lymphoid nodules area. At the same time, in subgroup 2V, a statistically significant decrease in lymphoid nodules area was observed by more than 2.5 times compared to subgroup 2KS. However, oral saponin administration on the day 14 statistically significantly increased the area of lymphoid nodules by 2.7 times in the subgroup of 2SV infected animals compared to the subgroup of 2V animals not treated with saponin.

Parameters of lymphoid nodules total area also naturally underwent significant changes. On the day 4, the subgroup of mice treated with oral saponin (KS) showed a statistically significant 3.9-fold increase in total lymphoid nodule area compared to the control subgroup (K) (Table 2). By the 14th day of follow-up, there was a 2.9-fold difference between the 2KS and 2K subgroups. The actual increase in lymphoid nodules total area in the KS and 2KS subgroups against the background of saponin administration amounted to 8.4 and 4.0 % of the total spleen area, respectively, due to a slight reduction in the area of the red pulp.

During the same follow-up period, despite a compensatory increase in total lymphoid nodules area, subgroup V showed a 7.4 % decrease in the percentage of relative

TABLE 1

ABSOLUTE VALUES OF THE SIZE OF LYMPHOID NODULES IN THE SPLEEN OF MICE INFECTED WITH INFLUENZA VIRUS AND TREATED WITH SAPONIN AT DIFFERENT INTERVALS OF THE EXPERIMENT

The average area (mm^2) of the lymphoid nodule on the day 4 of the experiment			
K* (*n* = 6)	**KS (*n* = 11)**	**V (*n* = 11)**	**SV (*n* = 11)**
0.08 ± 0.01	0.3 ± 0.03	0.2 ± 0.01	0.2 ± 0.01
	$p_K < 0.01$	$p_K < 0.01$ $p_1 < 0.01$	$p_K < 0.01$ $p_1 < 0.01$
The average area S of the lymphoid nodule on the day 14 of the experiment			
2K* (*n* = 6)	**2KS (*n* = 11)**	**2V (*n* = 11)**	**2SV (*n* = 11)**
0.08 ± 0.01	0.2 ± 0.01	0.03 ± 0.002	0.08 ± 0.004
	$p_K < 0.01$ $p_3 < 0.01$	$p_K < 0.01$ $p_2 < 0.01$	$p_2 < 0.01$ $p_3 < 0.01$

Note. p_K – statistical significance of the difference between the current group and the control; p_1 – statistical significance of the difference between the current group and KS; p_2 – statistical significance of the difference between the current group and 2KS; p_3 – statistical significance of the difference between the current group and 2V; * – control subgroup for the days 4 and 14 of the experiment.

TABLE 2

RELATIVE AND ABSOLUTE VALUES OF THE SIZES OF THE WHITE AND RED PULP SECTIONS OF THE SPLEEN OF MICE INFECTED WITH THE INFLUENZA VIRUS AND TREATED WITH SAPONIN AT THE DIFFERENT STAGES OF INFECTION

Groups	K* (n = 8)	KS (n = 11)	V (n = 11)	SV (n = 11)
day 4 of follow-up after infection with influenza virus				
Total area of lymphoid nodules, mm^2	1.7 ± 0.01	6.6 ± 0.01 $p_K \le 0.001$	4.4 ± 0.2 $p_K \le 0.001$	3.9 ± 0.2 $p_K \le 0.001$
Relative area of lymphoid nodules, % S of lymphoid nodules from the total S of the spleen	31.3	39.7	23.9	30.2
Total area of red pulp, mm^2	3.7 ± 0.2	9.9 ± 0.6 $p_K \le 0.001$	13.5 ± 0.8 $p_K \le 0.001$	8.8 ± 0.3 $p_K \le 0.001$ $p_1 \le 0.001$
The relative area of the red pulp, % S of the red pulp from the total S of the spleen	68.7	60.3	76.1	68.7
Total area of the spleen, mm^2	5.4 ± 0.2	16.7 ± 0.4	18.2 ± 0.4	12.8 ± 0.5
day 14 of follow-up after infection with influenza virus				
	2K* (n = 8)	**2KS (n = 11)**	**2V (n = 11)**	**2SV (n = 11)**
Total area of lymphoid nodules, mm^2	1.7 ± 0.01	4.9 ± 0.3 $p_K \le 0.001$	0.7 ± 0.03 $p_K \le 0.001$ $p_1 \le 0.001$	1.4 ± 0.02 $p_2 \le 0.01$ $p_3 \le 0.001$
Relative area of lymphoid nodules, %	31.3	35.3	9.8	18.6
Area of red pulp, mm^2	3.7 ± 0.2	8.9 ± 0.5 $p_K < 0.001$	6.9 ± 0.2 $p_K < 0.001$ $p_1 < 0.001$	6.2 ± 0.06 $p_k < 0.001$ $p_2 < 0.01$ $p_3 \le 0.05$
Relative area of red pulp, %	68.7	64.7	90.1	81.4
Total area of the spleen, mm^2	5.4 ± 0.2	13.8 ± 0.4	7.6 ± 0.02	7.6 ± 0.08

Note. p_K – statistical significance of the difference between the subgroup and the control; p_1 – statistical significance of the difference with subgroup V; p_2 – statistical significance of the difference compared to the subgroup SV; p_3 – statistical significance of the difference compared to subgroup 2V; * – control subgroup for the days 4 and 14 of the experiment.

lymphoid nodules area compared to the control subgroup. On the day 4 of the experiment, there is a statistically significant increase in the area of red pulp in subgroup V compared to the control by 3.6 times, which corresponds to an increase in the relative area by 7.4 %. The introduction of a corrector in the SV subgroup led to a statistically insignificant reduction in the total area of the white pulp by 8.8 % compared to subgroup V against the background of a statistically significant reduction in the area of the red pulp by 53.8 % during these experimental periods.

In subgroup 2V, there was a statistically significant reduction in the total area of lymphoid nodules by 16.4 % compared to the control, which was accompanied by a significant 21.4 % increase in red pulp area. From the day 4 to the 14 of the course of influenza infection, the total area of lymphoid nodules decreased by 5.8 times – from 23.9 to 9.8 %, respectively.

On the day 14 after the commencement of the experiment a statistically significant difference in the total area of lymphoid nodules was detected between the subgroups of infected mice receiving saponin (2SV) and those not receiving the substance (2V). The introduction of a corrector against the background of a viral infection contributed to a statistically significant increase in the total area of the lymphoid nodules by almost 2-fold and in the relative area by 8.8 % compared to the 2V subgroup.

DISCUSSION

Currently, infectious and particularly viral diseases have a significant impact both on morbidity and mortality patterns. In addition, the incidence of influenza, as well as other acute respiratory infections, is the cause of up to 50 % of all cases of temporary disability. The leading cause

of the high pathogenicity of influenza virus is the effect of its haemagglutinin superantigen proteins and NS1 and NS2 non-structural proteins that affect both central and peripheral immune system organs [21].

The reduced and inadequate immune status of the infected mice during this experiment is evidenced by the progressive marked depletion of lymphoid tissue in the largest organ of the peripheral part of the immune system, the spleen.

The immunological activity of the spleen is primarily reflected in the number and size of the lymphoid nodules. The periarterial zone of lymphoid nodules is known to be predominantly occupied by T-lymphocytes that form the cellular branch of immunity, whereas the marginal zone of lymphoid nodules is predominantly occupied by B-lymphocytes [22, 23].

The effect of the influenza virus on mice led to the emptying of lymphoid nodules and, consequently, to a decrease in the relative area of the white pulp of the spleen. At the same time, the total area of the organ increased mainly due to hyperaemia and swelling of the red pulp, whose elements could compensatively phagocytize the damaged red blood cells. On the day 4 after infection, there was a decrease in the germinal zone of the follicles, followed by progressive depletion of lymphoid tissue. By the day 14 of the infectious process, the germinative centers in lymphoid nodules, their boundaries, as well as their T-dependent periarterial zones were practically not visualized in most mice. This indicated the suppression of both the humoral and cellular branches of immunity.

Depletion of lymphoid tissue in the spleen of infected mice of both experimental groups could additionally be caused by lymphocyte apoptosis and a reduced rate of lymphoblast proliferation in response to influenza virus exposure, but the extent of programmed cell death was apparently lower in the group with therapeutic tauroside Sx1 administration at all follow-up periods. Morphometrically, a decrease in the size of the mantle and marginal lymphoid nodule zones and expansion of their germinal centres in subgroups SV and 2SV indicate a compensatory increase in the number of less differentiated cells against a decrease in the number of more mature lymphocytes and reduced red pulp hyperemia [23, 24].

Saponins are substances that have long been used in practical medicine due to the presence of a wide range of biological activity. Some types of saponins increase secretion of bronchial glands by stimulating the cough centre, and are therefore widely used as an expectorant. Ivy leaves extracts are the main components of Gedelix, Bronchipret, Prospan (Germany), Herbion (Slovenia) and are often used both for symptomatic treatment of acute respiratory diseases and to relieve symptoms of chronic bronchitis. However, the immunomodulatory effect of saponins has not been practically studied, since many of them have hemolytic activity [25–27].

The increase in lymphoid nodule area caused by the outgrowth of the marginal zone against the background of tauroside Sx1 administration indicates a probable activation of the humoral branch of immunity even in the absence of an infectious agent. Antigen-binding and antibody-synthesising cells are known to be most concentrated in the marginal zone of lymphoid nodules. In addition, a significant number of lymphoid nodules demonstrated lucent centres where B-lymphoblast proliferation was observed. At the same time, we did not detect any signs of the toxic effect of this saponin. The increase in the relative area of lymphoid nodules could also be the result of active lymphocyte migration from central organs of immunogenesis as a result of prolonged exposure to saponin, which may have contributed to the activation of antibody synthesis observed in our previous studies. Oral administration of saponin has previously been demonstrated to potentiate the immunopotentiating effect of intramuscularly administered subunit influenza vaccine. Vaccinated mice that received saponin at a dose of 200 μg per day after each immunisation showed a 2–10-fold increase in virus-specific antibody production against H1, H3 and haemagglutinin of influenza virus type B after 1–3 weeks [13].

From this fact, it can be concluded that oral administration of saponin tauroside Sx1 positively affects the morphofunctional transformations of the spleen (hyperplasia of the white pulp of the spleen, the appearance of new lymphoid nodules, moderate blood filling of the red pulp), which can be characterised as a quite significant immunostimulating effect.

Considering previous data about the ability of influenza viruses to cause immunosuppression by depleting lymphoid tissue, the use of phytoimmunostimulants in combination with antiviral drugs may contribute to a milder course of the viral infection.

CONCLUSION

Currently, the development and study of new effective domestic means for non-specific prevention of influenza that do not directly affect the virus is very relevant, which would exclude the possibility of resistance formation.

The experiment revealed that infection with the H1N1 influenza virus leads to compensatory activation of the animal's peripheral immune system by increasing the white pulp area as well as fullness of the red pulp, thereby increasing the overall size of the organ. On the 14th day following infection, there was a significant depletion of the white pulp, reflected by a reduction in the overall size of the lymph nodes and a loss of their zoning, which reflected the disappearance of the T- and B-lymphocyte germinal centers in them.

It follows from the data obtained that administration of the saponin tauroside Sx1 positively influences the functional activity of the spleen. This is demonstrated by an increase in the proportion of the white pulp of the spleen as a result of an increase in the size of the lymphoid nodules and their germinal centers. The above-described effects of Sx1 tauroside indicate the future prospects for further study and application of a number of saponins being used as immunomodulatory agents.

Conflict of interest

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

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EPIDEMIOLOGY

ASSESSMENT OF INDICATORS OF SPECIFIC HUMORAL IMMUNE AGAINST COVID-19 IN CHILDREN DURING THE DISTRIBUTION OF A NEW CORONAVIRUS INFECTION IN THE IRKUTSK REGION (2020–2021)

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ABSTRACT

Background. *There are many aspects of the development of immunity to the SARS-CoV-2 virus, that remain poorly understood, like the influence of age-related characteristics on the intensity of immunity and the course of the disease. Studies of the state of immunity are widely used, mainly in the adults. But questions of the patho- and immunogenesis in children remain unsolved. Determining the nature of seroconversion of antibodies to SARS-CoV-2 in this age group is important information for serological monitoring for targeted immunoprophylaxis of the population and forecasting the epidemic situation in the region.*

The aim. *Evaluation of the dynamics of seroprevalence of specific antibodies to SARS-CoV-2 in children of the Irkutsk region during the pandemic of a new coronavirus infection.*

Materials and methods. *Study was conducted among the child population of the Irkutsk region in the period June 2020 – December 2021 as part of the Rospotrebnadzor project to assess population immunity to SARS-CoV-2 in the population of the Russian Federation. The content of antibodies to SARS-CoV-2 was determined by ELISA using native commercial test systems.*

Results. *Population immunity among the child population of the Irkutsk region was characterized by an upward trend from 7.8 % at stage 1 to 98.4 % at stage 6 of the study. IgG to SARS-CoV-2 remained in 72.8 % and formed in 25.6 % of previously seronegative children. Antibodies were detected in 66.1 % of cases and persisted for up to 10–15 months after COVID-19 infection. The proportion of asymptomatic forms of infection among seropositive volunteers was 69.5 %, which determines the high intensity of the latent epidemic process.*

Conclusion. *The level of seroprevalence was 98.4 %. The current results of serological monitoring serve as a scientific basis for adjusting the list and scope of management decisions on the organization of preventive anti-epidemic measures, including vaccination.*

Key words: *children, COVID-19, SARS-CoV-2, humoral immunity, specific antibodies*

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ОЦЕНКА ПОКАЗАТЕЛЕЙ СПЕЦИФИЧЕСКОГО ГУМОРАЛЬНОГО ИММУНИТЕТА ПРОТИВ COVID-19 У ДЕТЕЙ В ПЕРИОД РАСПРОСТРАНЕНИЯ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ В ИРКУТСКОЙ ОБЛАСТИ (2020–2021 гг.)

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РЕЗЮМЕ

Актуальность. *В настоящее время многие аспекты развития иммунитета к вирусу SARS-CoV-2 остаются малоизученными, в том числе влияние возрастных особенностей на напряжённость иммунитета и течение заболевания – широкое распространение получили исследования состояния иммунитета преимущественно у взрослых, а вопросы пато- и иммуногенеза этой инфекции у детей остаются нераскрытыми. Определение характера сероконверсии антител к SARS-CoV-2 в данной возрастной группе является важной информацией при серологическом мониторинге для проведения таргетированной иммунопрофилактики населения и прогнозирования эпидемической ситуации в регионе.*

Цель. *Оценить динамику серопревалентности специфических антител к SARS-CoV-2 у детей Иркутской области в период пандемии новой коронавирусной инфекции.*

Материалы и методы. *В рамках проекта Роспотребнадзора по оценке популяционного иммунитета к SARS-CoV-2 у населения Российской Федерации проведены исследования среди детского населения Иркутской области в период с июня 2020 г. по декабрь 2021 г. Содержание антител к SARS-CoV-2 определяли методом ИФА, используя отечественные коммерческие тест-системы.*

Результаты. *Популяционный иммунитет среди детского населения Иркутской области характеризовался тенденцией к росту – от 7,8 % на 1-м этапе до 98,4 % на 6-м этапе исследования. IgG к SARS-CoV-2 сохранились у 72,8 % и сформировались у 25,6 % ранее серонегативных детей. После перенесённой инфекции COVID-19 антитела выявлялись в 66,1 % случаев и сохранялись до 10–15 мес. Доля бессимптомных форм инфекции среди серопозитивных волонтёров составила 69,5 %, что обусловливает высокую интенсивность скрыто протекающего эпидемического процесса.*

Заключение. *Уровень серопревалентности составил 98,4 %. Текущие результаты серологического мониторинга служат научной основой для корректировки перечня и объёмов управленческих решений по организации профилактических противоэпидемических мероприятий, включая вакцинацию.*

Ключевые слова: *дети, COVID-19, SARS-CoV-2, гуморальный иммунитет, специфические антитела*

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INTRODUCTION

COVID-19 was first reported in China in November 2019. Since then, this outbreak has rapidly developed into a global health emergency [1]. By May 2020, more than 4 million people had become infected with SARS-CoV-2. By January 2022, there were more than 350 million people infected with COVID-19 worldwide [2]. The disease is registered in almost all countries of the world, regardless of the climatic zone and the level of social and economic development. A significant proportion of asymptomatic forms of the infection characterizes the high intensity of the latently evolving epidemic process [3, 4]. The ubiquity of COVID-19 contributes to its progression among children. According to the literature, children account for up to 10 % of people infected with SARS-CoV-2 worldwide and 6–7 % in the Russian Federation [5–7]. One of the reasons is considered to be a significant frequency of asymptomatic forms of the infection, which remains unknown, but there is no doubt that this is due to the peculiarities of the child's immune system. It has been established that children with mild and/or asymptomatic course of the disease are able to produce specific SARS-CoV-2 antibodies (Abs), exerting a certain influence on the age structure of seroprevalence and herd immunity in general [8]. By the age of 4, more than 75 % of children are known to develop an immune response not only to seasonal, but also pathogenic coronaviruses [9].

The nature of the immune response to SARS-CoV-2 among children with various clinical manifestations from asymptomatic to MIS-C compared to the more common respiratory manifestations of COVID-19 among adults is unclear. Milder symptoms of the disease among children are the reason for low medical aid appealability, which in turn can lead to a decrease in detected cases of SARS-CoV-2 among children and adolescents. In addition, this age group with mild symptoms or with an asymptomatic course may contribute to the prevalence of COVD-19 among the population [10]. Therefore, the study of the virus-specific antibody reaction to SARS-CoV-2 among children and adolescents is important both for the development, adaptation and improvement of measures to control the incidence of COVD-19, and to address the issue of specific prevention of the child population.

THE AIM OF THE STUDY

Evaluation of the dynamics of seroprevalence of specific antibodies to SARS-CoV-2 in children of the Irkutsk region during the pandemic of a new coronavirus infection.

MATERIALS AND METHODS

The study was carried out as a part of the Rospotrebnadzor project to assess population immunity to SARS-CoV-2 among the population of the Russian Federation, taking into account the protocol recommended by WHO [11]. The studies were carried out according to a unified methodology developed by Rospotrebnadzor with the participation of the Saint Petersburg Pasteur Institute [12]. The work with volunteers was carried out in accordance with the ethical principles of the Declaration of Helsinki of the World Medical Association. The study was approved by the local Ethics committee of the Institute (Minutes No. 3 dated June 1, 2020, Minutes No. 7 dated November 11, 2021). Legal representatives of all volunteers got acquainted with the purpose and methodology of the study and signed an informed consent to participate in it.

A total of 384 randomly selected volunteers took part in the study, who passed a preliminary questionnaire and had a negative result of a study for the SARS-CoV-2 RNA by PCR. The same volunteers participated in each stage. The exclusion criteria during the immunological examination were: refusal of the patient's responsible representative to participate in the study, symptoms of acute respiratory infection with fever at the time of examination, the presence of acute or exacerbated chronic diseases 2 weeks before the study, lack of preparation required for the study.

The study of humoral immunity among children was conducted in 15 administrative territories of the Irkutsk region: from June 6 to July 7, 2020 – the 1st stage (384 subjects), from September 16 to September 25, 2020 – the 2nd stage (264 subjects), from December 7 to December 18, 2020 – the 3rd stage (249 subjects), from March 8 to March 14, 2021 – the 4th stage (212 subjects), from August 30 to September 4, 2021 – the 5th stage (224 subjects) and from December 13 to December 16, 2021 – the 6th stage (250 subjects).

In terms of gender of the volunteers participated in the study, the ratio of boys and girls was 1:1. All volunteers were divided into three age groups: 1–6, 7–13 and 14–17 years old.

The content of SARS-CoV-2 antibodies was determined by ELISA using a reagent kit for the analysis of blood serum or plasma for the presence of specific immunoglobulins G to the SARS-CoV-2 nucleocapsid produced by the State Research Center for Applied Biotechnology and Microbiology (Obolensk), as well as (at stages 5 and 6) using reagent kits for enzyme immunoassay detection of immunoglobulins G to the S-protein of the coronavirus – SARS-CoV-2-IgG-ELISA-BEST (Vector-Best LLC, Novosibirsk), and to the SARS-CoV-2 nucleocapsid proteins – SARS-CoV-2-IgG-Vector (State Research Center of Virology and Biotechnology VECTOR) and N-CoV-2-IgG PS (Saint-Petersburg Pasteur Institute).

The results were recorded qualitatively and were considered positive when the positivity index (PI) ≥ 1.1 was exceeded, the calculation of which was carried out according to the formula:

$$PI = (Sample\ OD) / critical\ OD\ (cut\ off),$$

where *Sample OD* is the measured optical density, and *critical OD (cut off)* is calculated in accordance with the instructions for the test system. For N-CoV-2-IgG PS, the results were considered positive when the quantitative IgG content exceeded ≥ 100 μg/ml.

Statistical data processing was carried out using variational statistics methods in Excel (Microsoft Corp., USA) and Statistica 6.0 (StatSoft Inc., USA). The probability le-

vel $p < 0.05$ was used to assess the statistical significance of the differences in the compared indicators. The normality of data distribution was determined using Kolmogorov – Smirnov test and Shapiro – Wilk test. The presented samples did not meet the criteria of normal distribution, therefore, nonparametric statistical methods for dependent samples (Wilcoxon test) were used in the comparative analysis. Pearson's chi-square (χ^2) test was used in contingency tables when comparing the level of seroprevalence at different observation periods. The data were expressed as median (Me) and interquartile range (Q25 %–Q75 %). Graphical data processing was performed using Excel (Microsoft Corp., USA).

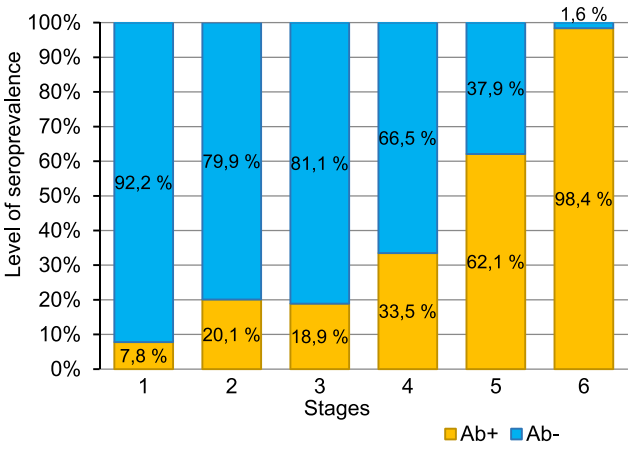

FIG. 1.
The level of seropositive volunteers among children at different stages of study

RESULTS AND DISCUSSION

Earlier, we showed that a low level of seroprevalence was formed in the Irkutsk region (stage 1 – 5.8 %; stage 2 – 12.1 %) at the onset of the epidemic outbreak and associated with a decrease in COVID-19 incidence. During the recrudescence period, seroprevalence reached 25.9 % (stage 3), which is 4.5 times more compared to stage 1 of the study [13]. It was found that herd immunity among the children of the Irkutsk region in the dynamics of observation tended to increase (Fig. 1) – from 7.8 % (stage 1) to 98.4 % (stage 6).

The number of seropositive volunteers was 30 out of 384 examined at stage 1, at stage 2 – 53 out of 264 subjects, at stage 3 – 47 out of 249 subjects, at stage 4 – 71 out of 212 subjects, at stage 5 – 139 out of 224 subjects, and at stage 6 – 246 out of 250 subjects (Fig. 1). Comparative analysis using χ^2 test showed statistically significant differences in the results at different stages of the study ($\chi^2 = 76.5$; df = 5; $p < 0.05$).

When comparing dependent samples of stages 1–4, Me ranged from 0.3 (0.2–0.5) at stage 1, 0.55 (0.3–0.9) at stage 2, 0.5 (0.3–0.8) at stage 3 and up to 0.7 (0.5–1.2) at stage 4 of the study (Fig. 2a). At stages 5 and 6, the quantitative test system N-CoV-2-IgG PS was used, so their comparison was carried out separately: Me was 140 (50–470) and 245 (40–610), respectively (Fig. 2b).

A high level of seroprevalence, which tended to increase, was established in Angarsk, Irkutsk, Usolye-Sibirskoye and Bratsk at all stages of the study. In December 2021, in some municipalities of the Irkutsk region (Angarsk, Taishet, Usolye-Sibirskoye), this parameter reached 100 % (Fig. 3). It is possible to estimate the reliability of statistical data in four localities (Bodaibo, Cheremkhovo, Bokhan, Ust-Ordynsky) only approximately, since the number of examined persons in these territories was less than 20 people.

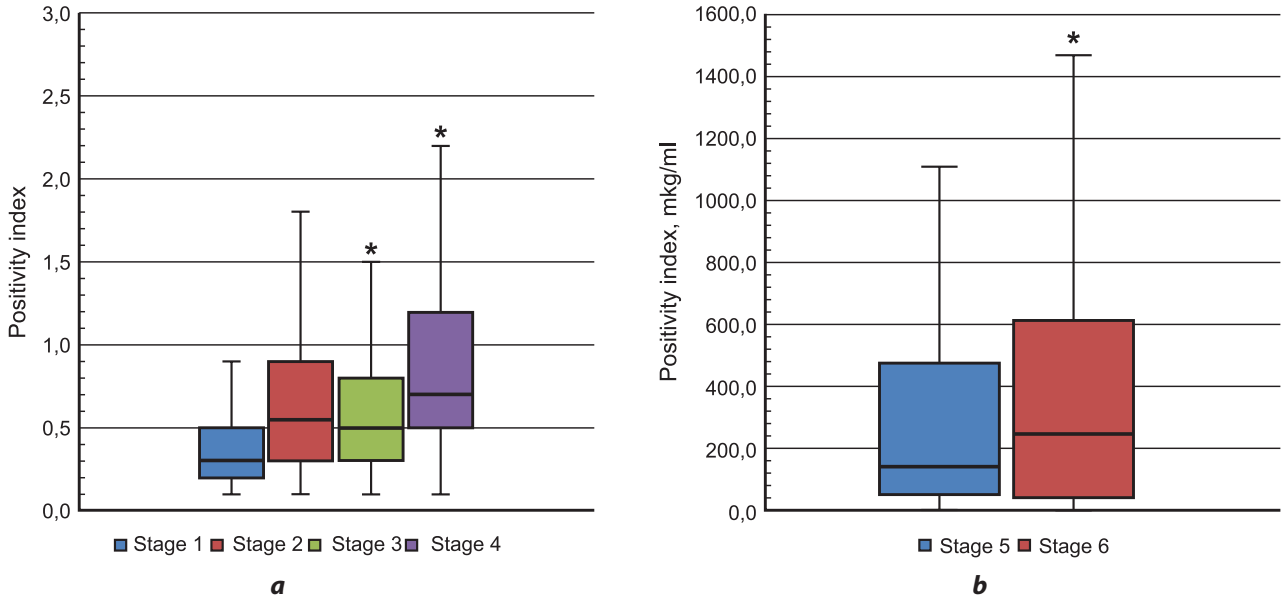

FIG. 2.
Positivity index values at each stage, Me (Q25 %–Q75 %): ***a*** *– stages 1–4, $p < 0.05$ compared to stage 1;* ***b*** *– stages 5 and 6, $p < 0.05$ compared to stage 5*

The maximum level of herd immunity among children at stages 1 and 2 of the study was noted in the group of 14–17 years (17 and 21 people respectively), at stages 3 and 4 – among preschool children (13 and 15 peo-

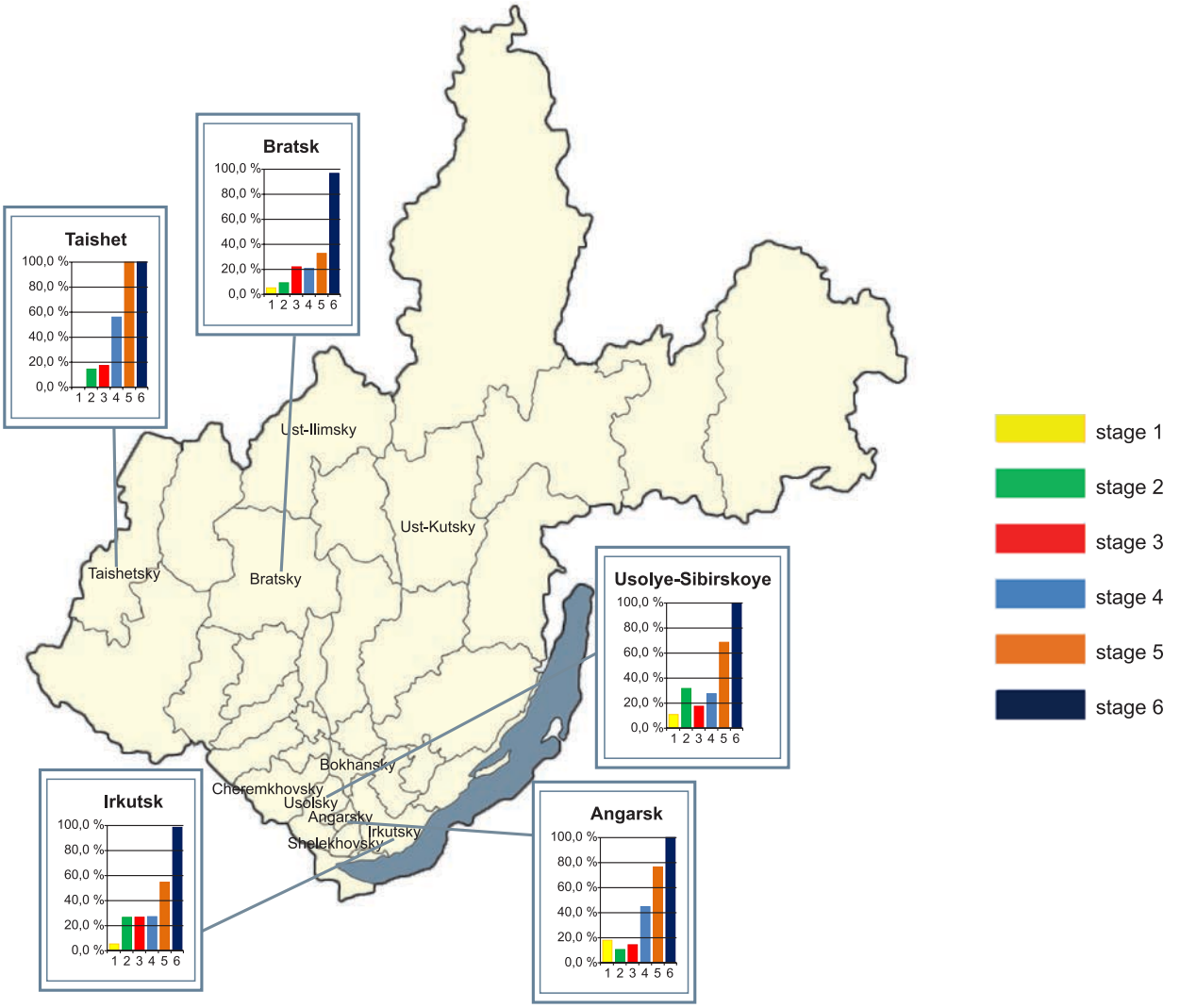

FIG. 3.

Indicators of seroprevalence among children in the territory of the Irkutsk region in the dynamics of observation

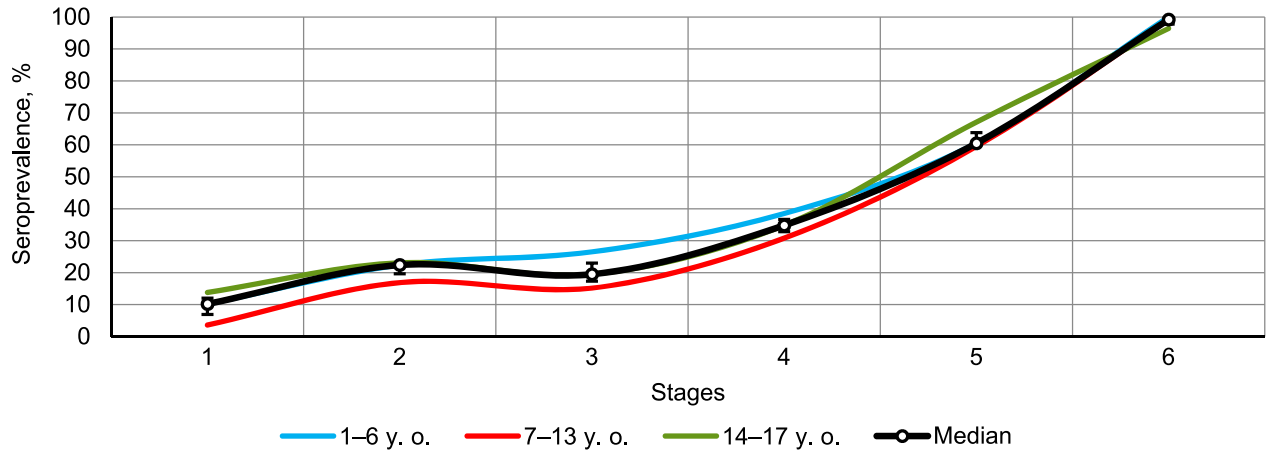

FIG. 4.

Dynamics of increasing seroprevalence to SARS-CoV-2 in children from stages 1 to 6. Colored lines – the process of changing the seroprevalence of children of the corresponding ages during the observation; the black bold line is the median; black vertical lines are the upper and lower quartiles (Q25 **%***–Q75 %)*

ple respectively), at stage 5 – in the group of 14–17 years (47 people), and at stage 6 – among preschoolers (38 people) (Fig. 4). During the study, the overall seroprevalence index among children increased by 12.6 times (from 7.8 to 98.4 %). 222 volunteers (52,9 %) with a primary negative result became seropositive. It is important to note that 57 seropositive (14.6 %) volunteers became seronegative 4–6 months after the detection of SARS-CoV-2 antibodies.

The percentage of children diagnosed with COVID-19 at stage 1 of the study was 0.52 % (2 people), at stage 2 – 1.5 % (4 people), at stage 3 – 7.6 % (19 people), at stage 4 – 7.1 % (15 people), at stage 5 – 12.1 %; (27 people) and at stage 6 – 15.2 % (38 people). At the same time, IgG to SARS-CoV-2 was detected in 50.0 % of volunteers at stage 1 of the study, at stage 2 – in 25 %, at stage 3 – in 42.1 %, at stage 4 – in 46.7 %, at stage 5 – in 81.5 %, and at stage 6 – in 100 %.

The analysis of seropositivity to SARS-CoV-2 at stage 6 of the study revealed a statistically significant preponderance of individuals with S-protein Abs (92.8 %) over the content of coronavirus nucleocapsid Abs (70.4 %). At the same time, the level of Abs to the studied antigens does not depend on age ($p > 0.05$). The study of the dynamics of antibody formation showed that Abs to Nc were detected on day 14 from the onset of the disease with a maximum increase in PI by day 185 and persisted up to day 432. Antibodies to SARS-CoV-2 S-protein were detected from day 7 after diagnosis and reached a peak by day 61, followed by a decrease to day 555.

Three possible types of seropositivity were identified among children based on the results of the study: twice positive (Nc+RBD), monopositive (Nc+ or RBD+) and negative (Nc– RBD–). The proportion of seronegatives was 1.6 %, the total proportion of RBD+ was 92.8 % and was statistically significantly higher ($p < 0.01$) than the Nc+ proportion – 70.4 %.

It is important to note that the total level of seroprevalence was 82.3 % during a comprehensive study of the herd immunity of the population of the Russian Federation (3,667 people), when calculating seropositivity to both antigens [14]. The results of a examination of persons with a history of COVID-19 (2020–2021) from the Irkutsk region indicate a long-term preservation of the humoral immune response among those who have had the infection. At the same time, the proportion of asymptomatic forms of infection among seropositive volunteers was 69.5 %, and in the Russian Federation – 76.9 %, which may indicate a high intensity of the latent epidemic process.

It was found that among the total cohort of examined volunteers aged 1–17 years, the number of children without a confirmed diagnosis of COVID-19, but having SARS-CoV-2 Abs, was 7.8 % at stage 1 (29 people), 20.1 % – at stage 2 (52 people), 18.9 % – at stage 3 (39 people), 33.5 % – at stage 4 (64 people), 62.1 % – at stage 5 (117 people) and 98.4 % – at stage 6 (208 people). No significant differences were found when assessing the correlation between the antibodies of volunteers with a confirmed diagnosis of COVID-19 and those who had this infection asymptomatically. A high proportion of seropositive children at stage 6 who had no manifestations of manifest infection (83.2 %) may indicate asymptomatic COVID-19 and the formation of full post-infectious immunity [14, 15], which may indicate a significant prevalence of undiagnosed cases of COVID-19 among children. Taking into account the proportion of cases of asymptomatic COVID-19, confirmed by the results of testing of seroprevalence of antibodies to Nc, the actual incidence of children is underestimated. In addition, children are less likely to have severe chronic pathology and are more likely to be infected by family members rather than vice versa, which may indicate a decrease in the virulence of the pathogen [15].

One of the most prominent manifestations of children's resistance to SARS-CoV-2 is the increased seroprevalence of Abs to pathogenic coronaviruses. An additional protective factor may be an increased level of seroprevalence of SARS-CoV-2 Abs, which is formed in response to the asymptomatic form of the course of coronavirus infection [14].

CONCLUSION

As part of the monitoring studies conducted for the first time among children aged 1–17 years, the fact of a widespread asymptomatic form of COVID-19 was confirmed, which does not exclude the formation of a humoral immune response. The proportion of seropositive to RBD and Nc was 92.8 and 70.4 %, respectively. Children have been found to be the main contributor to population immunity.

Antibodies were detected in 66.1 % of children with a PCR-confirmed diagnosis and persisted for up to 10–15 months to Nc and up to 15–18 months to RBD SARS-CoV-2 in the Irkutsk region after COVID-19 infection. Studies have shown that an increase in the level of Abs to RBD and Nc is accompanied by a decrease in the proportion of seroprevalence to SARS-CoV-2 antigens and levels the interage differences.

Thus, the results show a contribution of asymptomatic seroprevalent individuals to the level of humoral immunity to COVID-19 and a gradual decrease in the intensity of the epidemic of new coronavirus infection. The active formation of population immunity in combination with vaccination can serve as the main factor in ending the COVID-19 pandemic.

These results of serological monitoring can serve as a scientific basis for adjusting the list and volumes of epidemic control measures, making managerial decisions on the organization of preventive measures, including vaccination and forecasting the development of the epidemiological situation.

Conflict of interest

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

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HISTORY OF MEDICINE AND ANNIVERSARIES

HISTORY OF BIOMECHANICAL RESEARCH AT THE ILIZAROV CENTER (TO THE 100TH ANNIVERSARY OF ACADEMICIAN G.A. ILIZAROV)

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ABSTRACT

Background. *Biomechanical studies of both the Ilizarov apparatus itself and the physiological system "apparatus – limb" occupy a significant place in the history of the formation and elaboration of the Ilizarov method, developed in the middle of the last century at the Kurgan Research Institute of Experimental Traumatology and Orthopaedics (nowadays – the world-famous center named after its creator). The analysis of the history of biomechanical research in the formation of Ilizarov method is not without interest.*

The aim. *To analyse the history and stages of development of biomechanical research in order to substantiate the effectiveness of the Ilizarov method of transosseous osteosynthesis.*

Results. *The national medical industry did not produce the appropriate equipment for biomechanical research in the early 1970s. That is why a group of engineers was included into the Laboratory of Clinical Physiology and Biomechanics (established in 1971) of the Ilizarov Center, which created equipment for studying the processes in the tissues of the limbs and in the structure of the Ilizarov apparatus itself during its traction and compression impact on biological structures. The community of physicians, scientists and engineers made it possible to overcome a number of difficulties and problems. In their scientific publications and dissertations, the laboratory staff paid great attention to biomechanical research during transosseous osteosynthesis with the Ilizarov apparatus. At present, the staff of the Ilizarov Center continues the traditions established by G.A. Ilizarov. For the first time in our country, a computer 3D video analysis of the kinematics and kinetics of orthopedic patients gait was introduced; it was supplemented with embedded software for the preparation of a clinical report of human gait biomechanics.*

Conclusion. *The initial stage of the biomechanical research at the Ilizarov Center included the creation of the research equipment. Subsequently, the biomechanical studies carried out by the staff of the Center for almost half a century have shown an applied and functional result of the realization of general biological regularities of the Ilizarov's discovery. At present, the biomechanical research continues at a higher level with the use of modern high-tech equipment.*

Key words: *Ilizarov method, transosseous osteosynthesis, tension stress, Ilizarov effect, biomechanics*

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ИСТОРИЯ РАЗВИТИЯ БИОМЕХАНИЧЕСКИХ ИССЛЕДОВАНИЙ В ЦЕНТРЕ ИЛИЗАРОВА (К 100-ЛЕТИЮ АКАДЕМИКА Г.А. ИЛИЗАРОВА)

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РЕЗЮМЕ

Обоснование. *В истории становления и развития метода Илизарова, разработанного в середине прошлого века в Курганском НИИ экспериментальной травматологии и ортопедии, ныне – всемирно известном Центре, носящем имя своего создателя, значительное место занимают биомеханические исследования как самого аппарата, так и физиологической системы «аппарат – конечность». Интерес представляет анализ истории развития биомеханических исследований при становлении метода Илизарова.*

Цель. *Анализ истории развития биомеханических исследований для обоснования эффективности метода чрескостного остеосинтеза по Илизарову.*

Результаты. *Ввиду того, что в начале 1970-х годов соответствующего оборудования для проведения биомеханических исследований отечественная медицинская промышленность не выпускала, в состав лаборатории клинической физиологии и биомеханики Центра Илизарова (создана в 1971 г.) была введена группа инженеров, которая создавала аппаратуру для исследования процессов, происходящих в тканях конечностей и самой конструкции аппарата Илизарова при его тракционном и компрессионном воздействии на биологические структуры. Содружество врачей, научных сотрудников и инженеров позволило успешно преодолеть ряд трудностей и проблем. В научных публикациях и диссертациях сотрудники лаборатории уделяли большое внимание биомеханическим исследованиям в процессе выполнения чрескостного остеосинтеза аппаратом Илизарова. В настоящее время сотрудники Центра продолжают традиции, заложенные Г.А. Илизаровым. Впервые в нашей стране внедрён компьютерный 3D-видеоанализ кинематики и кинетики ходьбы у пациентов ортопедического профиля, который дополнен встроенным программным обеспечением для формирования клинического отчёта биомеханики ходьбы человека.*

Заключение. *Начальный этап развития биомеханических исследований в Центре Илизарова включал создание исследовательской аппаратуры. В последующем проводимые сотрудниками Центра в течение почти полувека биомеханические исследования показали прикладной, функциональный результат реализации общебиологических закономерностей открытия Г.А. Илизарова. В настоящее время биомеханические исследования осуществляются на более высоком уровне с использованием современного высокотехнологичного оборудования.*

Ключевые слова: *метод Илизарова, чрескостный остеосинтез, напряжение растяжения, эффект Илизарова, биомеханика*

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June 15, 2021 marked the 100th anniversary of the birth of Gavriil Abramovich Ilizarov, an outstanding scientist, academician of the Russian Academy of Sciences.

G.A. Ilizarov was an excellent organizer and inspirer of ideas, who gather around him a team of like-minded people and created a new method of treating injuries and diseases of the musculoskeletal system – the method of transosseous osteosynthesis.

Biomechanical studies of both the Ilizarov apparatus itself and the physiological system "apparatus – limb" occupy a significant place in the history of the formation, elaboration and justification of the Ilizarov method, developed in the middle of the last century at the Kurgan Research Institute of Experimental Traumatology and Orthopaedics (nowadays – the world-famous center named after its creator). In this work, we will focus on the analysis of the main stages of the development of biomechanical studies conducted and currently being conducted to physiologically determine the effect of the prolonged compression-distraction impact of the Ilizarov apparatus on the limb segment.

THE AIM OF THE WORK

To analyze the history and stages of development of biomechanical research in order to substantiate the effectiveness of the Ilizarov method of transosseous osteosynthesis.

After a historical insight and assessment of biomechanical approaches to the formation of the philosophy of the Ilizarov method from a biological standpoint the authors suggest that the reader evaluate the role of clinical biomechanics for improving diagnostic procedures and surgical interventions in orthopedics and traumatology.

In 1951 G.A. Ilizarov developed an apparatus which is an external fixation device consisting of a series of ring supports connected by threaded rods for fixing thin wires crosswise through the bone in a tensioned state [1]. This development reflected the concept of induction of local bone formation in a minimally invasive way, which provides for accurate reposition and stable fixation of bone fragments, exclusion of micro-mobility at their junction, sparing treatment of the soft tissues surrounding the bone, preservation of sources of osteogenesis. The theoretical basis of the distraction osteosynthesis method is the general biological regularity of stimulation of tissue regeneration and growth under the influence of tension stress, established on the basis of clinical experience and fundamental research and registered as a discovery [2]. After receiving good results of treatment of traumatological and orthopedic patients [3, 4] using the apparatus developed by G.A. Ilizarov [1], there were many uncertainties regarding the features of blood circulation and innervation of the operated limb segment, the condition of bones, surrounding soft tissues, muscle and joint function at various stages of treatment and rehabilitation.

To fill this gap, the Laboratory of Clinical Physiology and Biomechanics was established in 1971, headed by O.V. Tarushkin, Doctor of Biological Sciences (Fig. 1a). The national medical industry did not produce the appropriate equipment for biomechanical research in the early 1970s. That is why a group of engineers was included into the Laboratory, which created equipment for studying the processes in the tissues of the limbs and in the structure of the Ilizarov apparatus itself during its traction and compression impact on biological structures. The community of physicians, scientists and engineers made it possible to achieve a number of objectives.

a

b

FIG. 1.

Heads of the Laboratory of Clinical Physiology and Biomechanics: ***a*** *– Oleg V. Tarushkin, Dr. Sc. (Biol.) (1971–1977);* ***b*** *– Vladimir A. Shchurov, Dr. Sc. (Med.), Professor, High Level Certificate Physician (1977–2008)*

The photo (Fig. 2) shows the meeting of the Ilizarov Center staff with Wolf Messing in December, 1971. Among those present we can see Oleg V. Tarushkin and Vladimir A. Shchurov (indicated by white arrows), who at different times headed the Laboratory of Clinical Physiology and Biomechanics.

The most urgent tasks that the Center's staff faced in the early 1970s were the development of a safe method of drilling bone tissue with a wire and the evaluation of the mechanism of fixation of the wires when they pass through the cortical plate of the bone. On the instructions of G.A. Ilizarov, the Laboratory of Clinical Physiology and Biomechanics developed a method for measuring the temperature during bone drilling with a wire at any depth during its insertion [5] (Fig. 3).

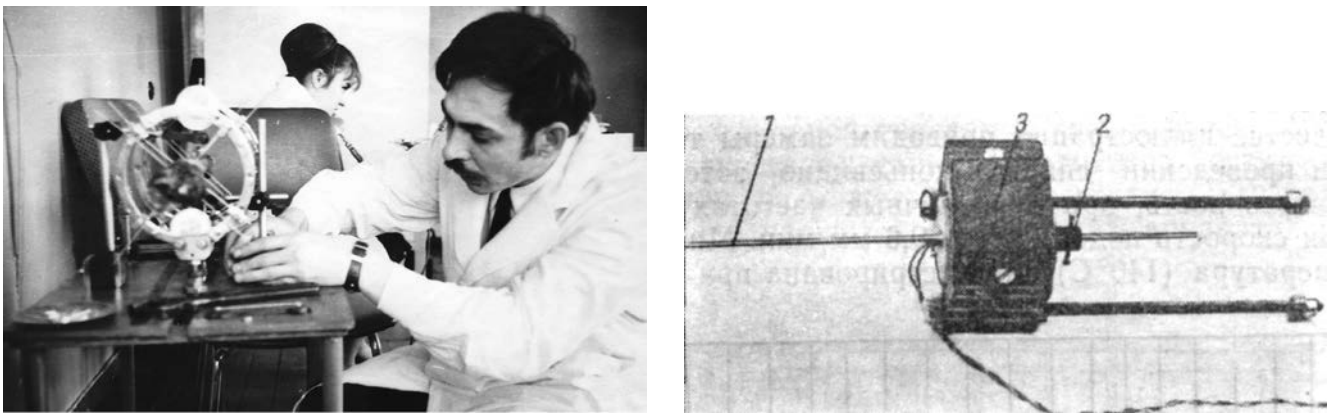
The effect of the tension forces of the wires on the rigidity of the bone fragments fixation in the apparatus was thoroughly studied. The rigidity of Kirschner wires in various configurations of the Ilizarov apparatus was analyzed (Fig. 4, 5) [6].

G.A. Ilizarov also set the task for the laboratory staff to evaluate the dynamics of deformation properties of a regenerated bone during the treatment.

The laboratory has developed an *in vivo* method for measuring the axial motion of bone fragments under conditions of compression osteosynthesis by the Ilizarov apparatus. Tensometric sensors were used to analyze changes in the distance between the wires coming out of the bone proximally and distally from the fracture site under conditions of axial functional load with different forces. This method allowed to evaluate the rigidity of the "apparatus – limb" system and the rheological properties of distraction regenerated bone [7, 8] (Fig. 6).

An original biomechanical track "Podographometer" was constructed in order to study the functions of the musculoskeletal system. This device allowed to automatically register and process the temporal indicators of gait [9, 10]. A quantitative assessment of gait was made. The obtained data were subjected to statistical development on the computer "NAIRI-2" according to the program developed by E.B. Smirnov –

FIG. 2.
Meeting of the staff of the Ilizarov Center with Wolf Messing (December 20, 1971)



a ***b***

FIG. 3.
a *– engineer V.A. Nemkov at work,* ***b*** *– thermocouple wire for measuring the temperature during bone drilling (1 – wire; 2 – collector; 3 – case) [5]*

one of the employees of the Center [11]. The functional results of treatment and the effectiveness of rehabilitation measures were evaluated in orthopedic and trauma patients treated by the Ilizarov method, using Podographometer.

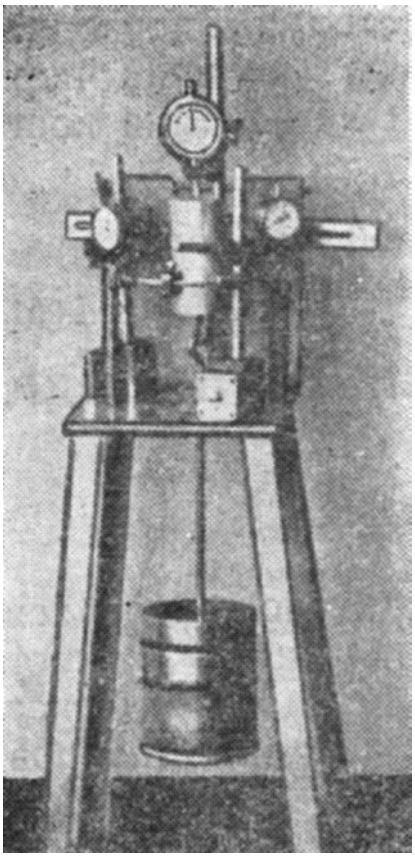


FIG. 4.
Stand for measuring wire deflections [6]

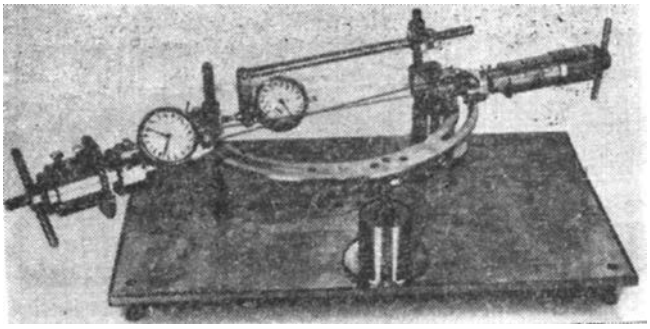
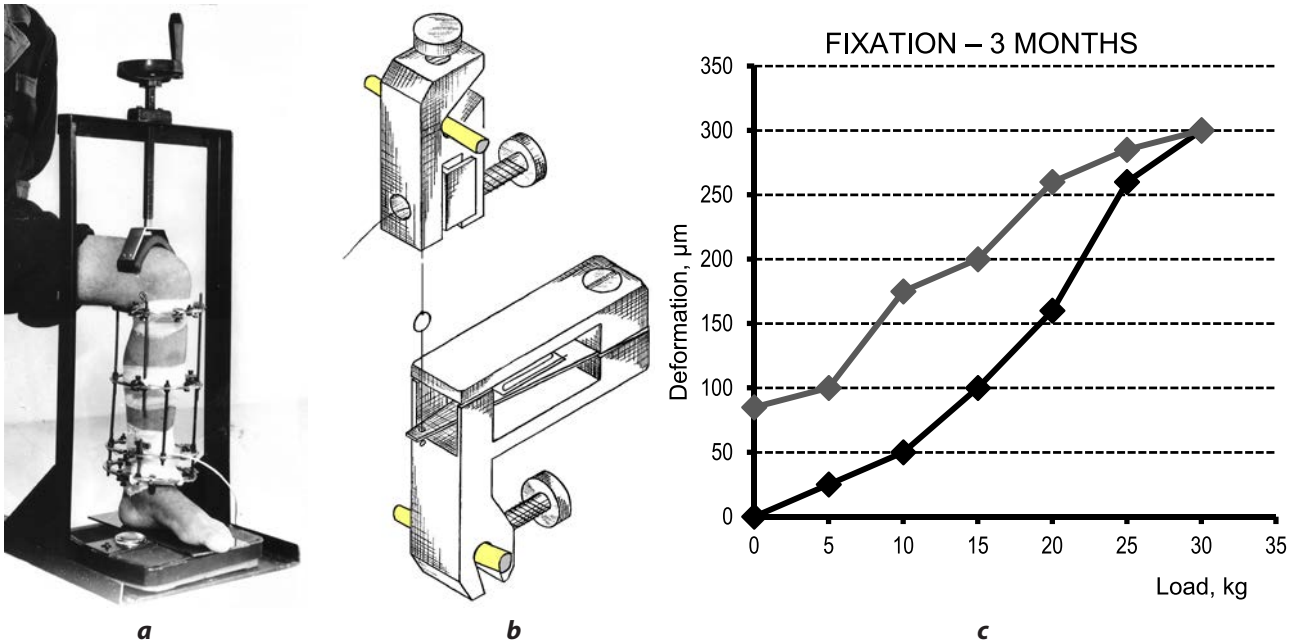


FIG. 5.
Stand for measuring the deformation of the Ilizarov apparatus parts under different loads [6]

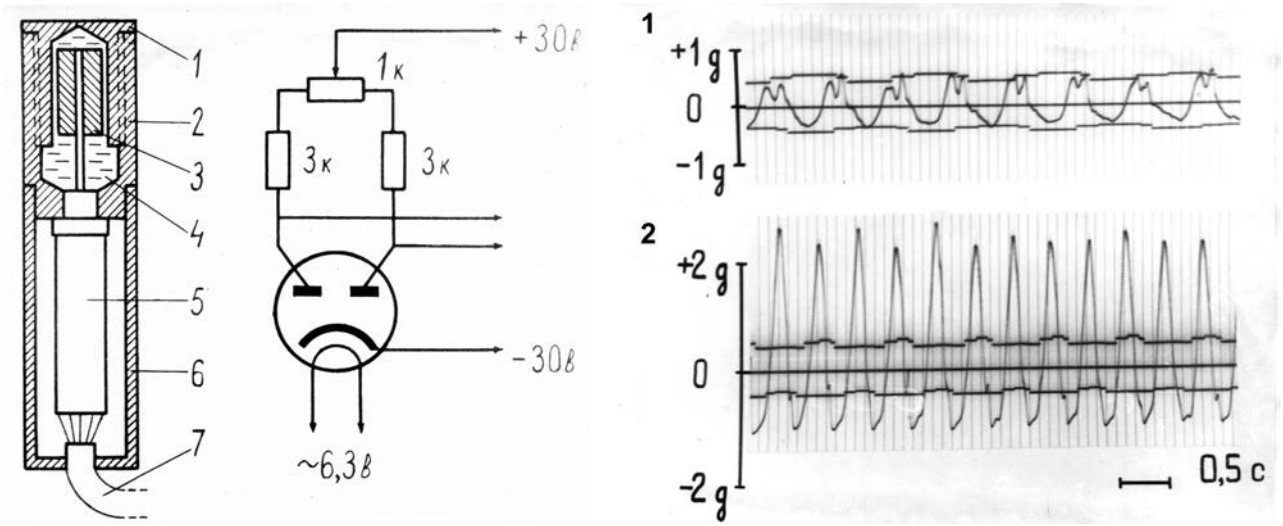
A mechatronic pedometer was developed to assess the motor activity of patients with injuries and diseases of the musculoskeletal system [12]. The design of the accelerometer sensor is proposed (Fig. 7) to determine the qualitative and quantitative characteristics of kinematics of the gait of a healthy and a diseased person, to assess the dynamics of the locomotor act under the influence of the treatment and functional rehabilitation [13]. Approaches in determining the biomechanics of movement, which are used in modern electronic gadgets, were applied in these designs.

Evaluation of the functional load on the lower limb during transosseous osteosynthesis by the Ilizarov apparatus was carried out using an original technique (cargometry) and a device developed at the Center. Clinical approbation of this technique carried out by Boris I. Kudrin, Candidate of Medical Sciences, allowed to estimate the supporting capabilities of the limb during walking in the course of treatment with the Ilizarov apparatus by the integral values of time and strength parameters of the interaction of the limbs with the biomechanical track [14–16] (Fig. 8).



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

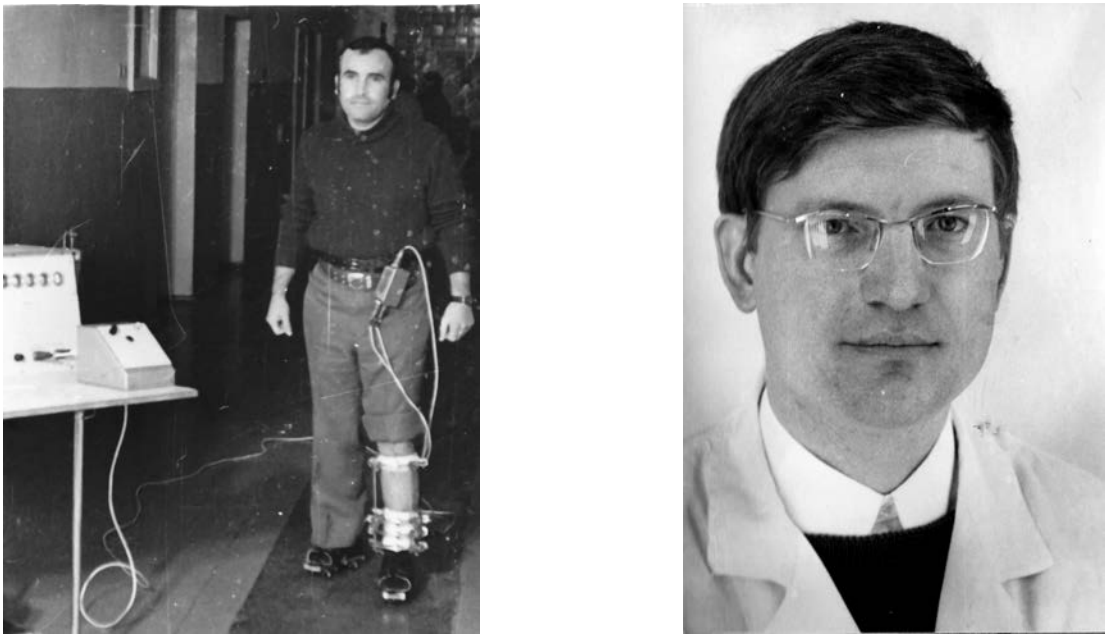
FIG. 6.
*Determination of the micromotion of bone fragments: **a** – the unit for determining the micromotion of bone fragments; **b** – tensometric sensor mounting; **c** – diagram for determining the rheological properties of distraction regenerated bone [7, 8]*



a *b* *c*

FIG. 7.

*Accelerometer device (**a**), the scheme of its inclusion in the measuring circuit (**b**) and the accelerogram (**c**) during walking (1) and running (2) of a healthy subject [13]*



a *b*

FIG. 8.

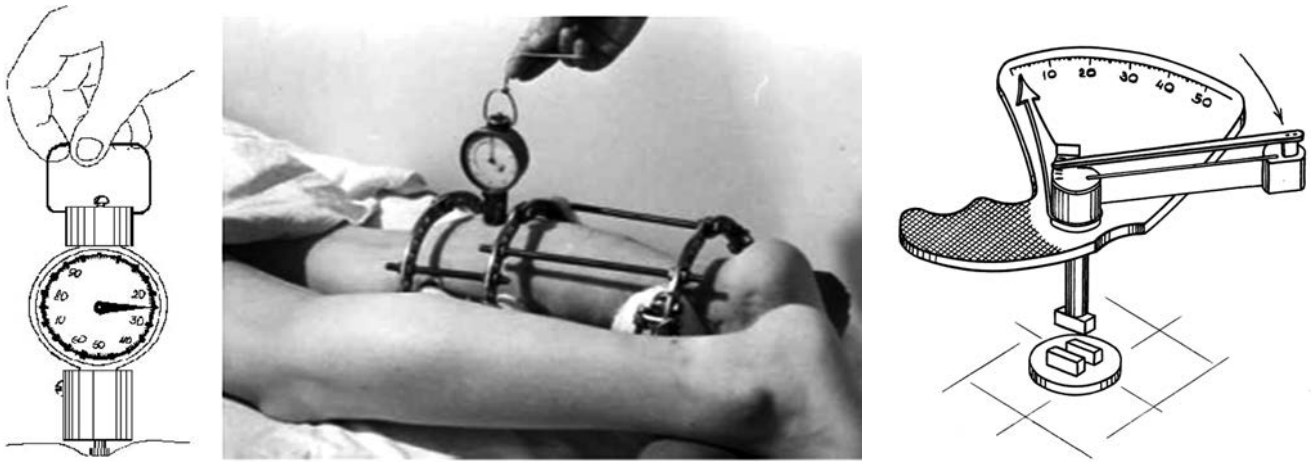
***a** – the patient during the evaluation of the lower limb functional load during transosseous osteosynthesis with the Ilizarov apparatus [16];*
***b** – Boris I. Kudrin, Cand. Sc. (Med.)*

In the late 1970s, G.A. Ilizarov formulated the concept "The stimulating effect of tension stress on tissue growth and regeneration" [2]. Obviously, there was a need for careful development of a theoretical justification and quantitative confirmation of the provisions of this concept [17]. The laboratory staff developed original designs for studying skin elasticity (elastometer) and myogenic tonus (tonometer) (Fig. 9) [18–20].

Studies of healthy children have shown that the values of skin and muscle elasticity increase with natural longitudinal growth of a limb. Patients with retardation in growth of limb segments after surgical lengthening have the same indicators of muscle and skin elasticity as healthy people with the same length of the corresponding limb [21–23].

In the process of development and continuous improvement of the Ilizarov method, there was a need for an accurate quantitative assessment of the muscle function (strength) of the operated limb. Professor V.A. Shchurov, Doctor of Medical Sciences, together with the laboratory staff, created original dynamometric stands [24, 25]. Later these methods were improved [26, 27] and published [28, 29] (Fig. 10).

At the stage of introduction of outpatient treatment of orthopedic and trauma patients into clinical practice (late



a *b* *c*

FIG. 9.

A tonometer, an original device for studying biomechanical properties of muscles [18, 20]: ***a*** *– external view (scheme);* ***b*** *– the process of measuring the myogenic tonus;* ***c*** *– an elastometer, the original design (scheme) for the assessment of skin elasticity [19]*

a *b* *c*

FIG. 10.

Dynamometric stands: ***a*** *– an external view of the dynamometric stand for studying femoral muscle strength;* ***b*** *– the process of measuring;* ***c*** *– studying the lower leg muscle strength during treatment with the Ilizarov apparatus*

1980s – early 1990s), it was necessary to develop functional criteria for transferring inpatient patients to this treatment regimen. The laboratory staff carried out comprehensive work, including the study of daily locomotor activity, linear velocity of movement, asymmetry of functional load on the limb, energy cost of locomotion.

When deciding on the advisability and timeliness of transferring inpatient patients to the outpatient treatment regimen, assessing clinical condition and analyzing their social and living conditions, it was proposed to use the following physiological criteria: value of the asymmetry of loading of the lower extremities when standing without additional support – no more than 20 %; gait velocity – at least 2 km/h; daily locomotor activity – at least 2000 m; pulse rate during walking – no more than 100–110 bpm [30].

In the 1990s–2000s, a number of PhD theses and doctoral dissertations were completed and successfully defended in the Laboratory of Clinical Physiology and Biomechanics, in which the biomechanical research was one of the leading topics.

In his doctoral dissertation (1993) V.A. Shchurov substantiated the physiological mechanism of the stimulating effect of tissue stretching that occurs during natural growth and limb lengthening according to Ilizarov [31].

In the PhD thesis of T.I. Dolganova (1993), the features of hemodynamics, biomechanical properties of tissues and the functional state of patients with leg bone defects in the treatment according to the Ilizarov method were analyzed [32]. In the work of E.N. Shchurova (1996), the role of locomotor activity in preserving the reserves of functional adaptation of blood circulation in patients with obliterating lesions of arteries of the lower extremities during treatment according to the method of G.A. Ilizarov was determined [33–35]. In the dissertation of T.I. Menshikova (1997), a physiological analysis [36–38] of the effect of lengthening of the lower extremities on the indicators of loco-

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

FIG. 11.

*Computer analysis of gait parameters using modern video analysis systems: **a** – general view and arrangement of the gait analysis laboratory; **b** – the process of examining the patient by the laboratory staff; **c** – external view of a patient before the examination with reflective markers and electromyographic sensors*

motor activity of patients with achondroplasia was given [39]. The work of D.V. Dolganov (1997) highlights the role of tissue hydration in the vegetative support of the limb during transosseous osteosynthesis [40, 41]. In her dissertation (2002) L.Y. Gorbacheva analyzed the influence of the functional load of the injured limb on the restoration of muscle contractility and reparative bone regeneration in the treatment of trauma patients according to the method of G.A. Ilizarov [42].

The doctoral dissertations continued the development of theoretical justification and quantitative confirmation of the general biological regularities of G.A. Ilizarov's discovery [2]. The physiological studies contributed to a deeper understanding of the mechanisms of "The Ilizarov effect", its biomechanical and structural components, illustrated the applied functional result of the implementation of the regularities of G.A. Ilizarov's discovery [43–45].

The results of many years of research and the developed designs were summarized, improved and published in the second London edition of "Biomechanics and Biomaterials in Orthopedics" in 2016 (Springer) [46].

The Ilizarov Center continues to carry out biomechanical research, the traditions of which were established more than half a century ago.

One of the areas of research is the search for diagnostic criteria for assessing disorders of the vertical orientation of the spine. The laboratory staff began to use a prolonged topographic examination to assess the stereotype of postural activity of the spine and its kinematic lability. Functional tests with correction and hypercorrection of limb shortening were introduced, which provided differentiated diagnostics of structural and functional scolioses, determination of the necessity and value of optimal correction of leg length inequality. Topographic analysis of stereotypes of postural activity and kinematic lability of the spine allowed to study pathogenetic conditions under which functional scolioses become structural ones [47–49].

In the Ilizarov Gait Analysis Laboratory of the National Ilizarov Medical Research Center for Traumatology and Orthopaedics, a computer analysis of gait parameters in orthopedic patients is performed on a 7-meter track using modern systems of video analysis of the kinetics and kinematics of human movements [50] (Fig. 11).

The data were recorded by 8 Qualisys 7+ optical cameras (Sweden) with passive marker motion capture technology, synchronized with 6 KISTLER dynamometers (Switzerland) and supplemented with 16 Noraxon wireless EMG sensors (USA). Kinematics and kinetics analysis are carried out in Qualisys and Visual 3D (C-Motion) with automated calculation of indicators. The biomechanical characteristics of the "stair movement" are determined using the "virtual platform lift" option. The research results are reflected in modern publications [51–53]. Our Center has developed embedded software for the preparation of the report on examination results in the form of digital data in tables complemented by diagrams [54].

CONCLUSIONS

The initial stage of the development of biomechanical research at the Ilizarov Center included the creation of equipment for studying the processes in the tissues of the limbs and the Ilizarov apparatus itself during its traction and compression impact on biological structures.

The community of doctors, scientists and engineers of the Laboratory of Clinical Physiology and Biomechanics (established in 1971) made it possible to develop devices and methods for measuring the temperature during bone drilling with a wire; assessing the effect of the tension forces of the wires on the rigidity of bone fragments fixation in the apparatus; studying axial micromotion of bone fragments and rheological properties of regenerated bone; studying the functions of the musculoskeletal system (original biomechanical track, mechatronic pedometer, accelerometer); studying biomechanical properties of muscles (myotometer, dynamometric stands); analysis of skin elasticity (elastometer).

In the following years, complex biomechanical studies contributed to the theoretical justification and quantitative confirmation of the stimulating effect of tension stress on tissue growth and regeneration (the Ilizarov effect), have shown the applied, functional result of the realization of general biological regularities of the Ilizarov's discovery.

Currently, the Ilizarov Center has switched to high-tech methods of biomechanical research, developed embedded software for the preparation of a report in the form of digital data of a computer 3D video analysis of gait complemented by diagrams.

Conflict of interest

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

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