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Dear colleagues!

I am delighted to introduce you to the new issue of the Extreme Medicine Journal released by the Federal Medical Biological Agency of Russia. The current issue features original research articles on experimental and clinical medicine, organ transplantation, epidemiology, physiology, and public health management. A few publications expectedly focus on the novel coronavirus infection, the ongoing threat facing the world, including a clinical review looking at COVID-19 from an ophthalmologist's perspective and a study proposing new methods to manage the sequalae of the infection.

For all of us, 2020 has been a year of change — and the Journal has changed too. This is the second issue with an updated layout, published in two languages. Our website can now boast better usability.

The ending year has posed serious challenges to public health systems all over the world. However, despite the hardships, it has been very productive for the medical science. The pandemic has brought into focus new vectors and trends in public health and medical science that are yet to be thoroughly investigated.

From the outset of the pandemic, FMBA of Russia has been on the frontline in the fight against COVID-19, mobilizing the resources and potential of its healthcare institutions. The experience gained is invaluable, and we will share it with you in upcoming issues.

Dear friends, we are facing great challenges in every aspect of our work. May the new year 2021 be the time of discoveries and breakthroughs in public health and medical science.

My best wishes of good health, happiness and well-being to you and your families.

Veronika Skvortsova

USING EXPERIMENTAL EX VIVO MODELS TO DEVELOP COVID-19 PATHOGENETIC THERAPY AND COMPLICATIONS PREVENTION AGENTS

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COVID-19 is a disease characterized by damage to the lower respiratory tract, development of the acute respiratory distress syndrome, in severe cases — multiple organ failure, including acute heart failure and cardiomyopathy. This study aimed to evaluate the effectiveness of the developed COVID-19 pathogenetic therapy and complications prevention agents using the *ex vivo* isolated lung and heart models. Isolated organs of white rats were used for the research; the dynamics of functional indicators were analyzed. An amino acid-peptide complex (APC) from a thermally treated milk protein hydrolyzate was used as the experimental COVID-19 pathogenetic therapy and complications prevention agent. Introduction of the APC to the isolated cardiopulmonary complex perfusate slowed down development of pulmonary edema in the experimental group; the organ's weight was 1.5 times less than in the control group ($p = 0.0158$). We have also registered an airway resistance downtrend. APC supported contractile activity of the isolated myocardium suffering ischemia-reperfusion: the growth of the left ventricular end diastolic pressure was 34% smaller than that registered in the control group ($p < 0.05$). The APC's cardioprotective effect relies on the endothelium-dependent mechanisms. The *ex vivo* method is highly informative. It allows assessing reactivity of the isolated organs exposed to biologically active substances and determining the possibilities of compensating for functional changes.

Keywords: isolated heart, ischemia, isolated lung, pulmonary edema, COVID-19

Author contribution: Laptev DS — experimental part, information collection, data processing; Petunov SG — data processing and interpretation, general guidance; Nechaykina OV — experimental part, information collection; Bobkov DV — data processing; Radilov AS — data processing and interpretation.

Compliance with ethical standards: all work with animals was carried out in conformity to the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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ИСПОЛЬЗОВАНИЕ ЭКСПЕРИМЕНТАЛЬНЫХ МОДЕЛЕЙ EX VIVO ДЛЯ РАЗРАБОТКИ СРЕДСТВ ПАТОГЕНЕТИЧЕСКОЙ ТЕРАПИИ И ПРОФИЛАКТИКИ ОСЛОЖНЕНИЙ COVID-19

Д. С. Лаптев ✉, С. Г. Петунов, О. В. Нечайкина, Д. В. Бобков, А. С. Радилов

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COVID-19 — заболевание, характеризующееся поражением нижних дыхательных путей, формированием острого респираторного дистресс-синдрома, в тяжелых случаях — развитием полиорганной недостаточности, в том числе острой сердечной недостаточности и формированием кардиомиопатии. Целью работы было оценить эффективность разрабатываемых средств патогенетической терапии и профилактики осложнений COVID-19 с использованием моделей изолированных легких и сердца *ex vivo*. Исследования проводили на изолированных органах белых крыс с анализом динамики функциональных показателей. В качестве средства экспериментальной патогенетической терапии и профилактики осложнений COVID-19 использовали аминокислотно-пептидный комплекс (АПК) из термически обработанного гидролизата молочного белка. Добавление АПК в перфузат изолированного сердечно-легочного комплекса способствовало снижению скорости формирования отека легких в опытной группе, при этом масса органа была в 1,5 раза меньше, чем в контрольной группе ($p = 0,0158$). Показана тенденция к снижению сопротивления дыхательных путей. Применение АПК способствовало поддержанию сократительной активности изолированного миокарда в условиях ишемии-реперфузии: увеличение конечного диастолического давления было на 34% меньше по сравнению с контролем ($p < 0,05$). Кардиопротекторное действие АПК обусловлено эндотелий-зависимыми механизмами. Метод *ex vivo* обладает высокой информативностью, позволяет провести оценку реактивности изолированных органов при действии биологически активных веществ и определить возможности компенсации функциональных изменений.

Ключевые слова: изолированное сердце, ишемия, изолированное легкое, отек легких, COVID-19

Вклад авторов: Д. С. Лаптев — экспериментальная часть, сбор информации, обработка данных; С. Г. Петунов — обработка и интерпретация данных, общее руководство; О. В. Нечайкина — экспериментальная часть, сбор информации; Д. В. Бобков — обработка данных; А. С. Радилов — обработка и интерпретация данных.

Соблюдение этических стандартов: все работы с животными выполняли с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей.

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SARS-CoV-2 belongs to the group of highly pathogenic coronaviruses that cause a clinical syndrome known as the novel coronavirus infection (COVID-19). In severe cases, COVID-19 is a systemic disease associated with lower respiratory tract damage, development of pneumonia, acute respiratory distress syndrome (ARDS), cytokine storm and growing levels of the heart damage biomarkers [1].

Mainly, the infection caused by SARS-CoV-2 manifests in the form of respiratory failure, which is the result of SARS-CoV-2 penetrating into target cells that have angiotensin-converting enzyme 2 (ACE2) receptors. In particular, this enzyme is expressed on alveolar type II pneumocytes (AT2), in enterocytes of the small intestine's epithelium, in the vascular endothelium and in cardiomyocytes. In the lungs, SARS-CoV-2

causes desquamation of type II and type I pneumocytes, which translates into alveolar dysfunction, lower surfactant levels, accumulation of liquid exudate in the alveolar space and pulmonary edema, which dramatically reduces the effectiveness of external respiration. This stage is characterized by moderate constitutional symptoms and determines the initial response of innate immunity [2].

From the point of view of morphological changes, SARS-CoV-2 patients may have edema with pleural effusion, focal hemorrhages and mucopurulent secretion in the airways. A distinctive histological feature observed at later stages is the diffuse lung lesion with fibrin exudation in the alveolar spaces and septal and alveolar fibrosis [3, 4]. Even if the viral load decreases, stronger inflammatory response leads to systemic inflammation [1], which is characterized by multiple organ failure and the increasing number of key inflammation markers.

The development of myocarditis associated with COVID-19 and going without signs of direct viral infiltration indicates that the heart is one of the targets of systemic inflammation [5], with the biomarkers of heart damage and electrocardiographic abnormalities in accord with the elevated levels of inflammatory markers [6, 7].

Either on its own or in combination with respiratory failure, heart damage and failure were the cause of death associated with COVID-19 in 40% of cases registered in one of the cohorts traced in Wuhan, PRC. The risk of death associated with acute myocardial injury was more significant than such factors as age, diabetes mellitus, chronic lung disease or previous cardiovascular disease [6, 8, 9]. Thus, damage to the heart is both a predominant type of COVID-19 complication and, as it seems, one of the complications to be predicted.

The mechanisms behind damage to the heart induced by SARS-CoV-2 remain virtually unstudied, however, it is likely they include both direct infection of the myocardium and the consequences of respiratory failure, hypoxemia, and systemic inflammatory response. Signaling pathways associated with ACE2, which are highly expressed in the heart, may also play a role in myocardial damage [9].

The significant severity of COVID-19 clinical manifestations and consequences thereof make it a priority to develop drugs to effectively prevent the development of severe conditions that translate, first of all, into acute damage to lungs and heart. Preclinical evaluation of the effectiveness of pharmacological agents requires use of experimental models that allow assessing the dynamics of parameters of the target organs' functional activity, such assessment adequate to the tasks set. *Ex vivo* experiments with isolated organs are highly informative: they allow objective registration of the organs' activity parameters.

The purpose of this work was to demonstrate the possibility of using isolated organs — heart and lungs — as models in preclinical studies of the effectiveness of developed COVID-19 pathogenetic therapy and complications prevention agents.

METHODS

The subjects were isolated lungs and hearts of white male Wistar rats weighing 280–360 g, obtained from the Rappolovo laboratory animal nursery (Leningrad region). The experimental animals were kept in conditions conforming to the Sanitary Rules for the Design, Equipment and Maintenance of Experimental Biological Clinics (vivariums). For research, we used healthy sexually mature animals quarantined for at least 10–14 days. The controlled microclimate parameters in the rooms where the animals were kept were temperature of 20 ± 1 °C, relative air humidity of 60 ± 5%. We have also controlled the quality of the bedding material. The animals received standard pellet feed. The lighting regime for the rooms containing experimental animals was 12 h of day and 12 h of night.

Isolated lung model

In addition to the gas exchange function, the model allows assessing the contribution of external respiration to metabolism of biologically active substances and the associated microcirculation in the pulmonary circulation. This model can be used to study pathogenesis of the bronchopulmonary diseases, including development of the pulmonary edema, as well as to assess the effectiveness of symptomatic and pathogenetic therapeutic agents designed for the respiratory system.

The experimental animals were anesthetized with a 20% urethane solution injected intraperitoneally and subcutaneously, 1.2 g per a kg of animal body weight. After a midline laparotomy, we injected heparin into the inferior vena cava to prevent thrombogenesis. Then we cannulated the trachea at the thyroid gland level and put the animal on a mechanical ventilator (respiratory rate — 50 min^{-1}, tidal volume — 1.7 ml, minute respiration volume — 85 ml/min).

After opening the thoracic cavity, we cannulated the pulmonary artery and connected to the peristaltic pump of the experimental rig; thus, we simulated pulmonary circulation. After removal of the heart-lung complex from the chest, we weighed it and placed it in Isolated Lung System (Radnoti; Ireland) chamber (Fig. 1), where the temperature and humidity were kept optimal for the object studied.

At the end of the first 30 min (stabilization period), the tidal volume was gradually, within 15 minutes, increased from 1.7

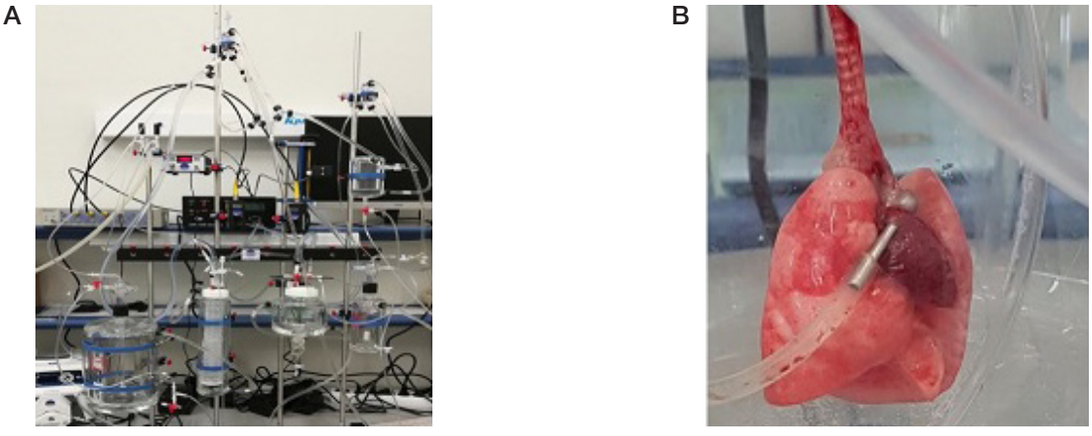

Fig. 1. Isolated lung system. **A**. General view of the installation. **B**. Heart-lungs complex

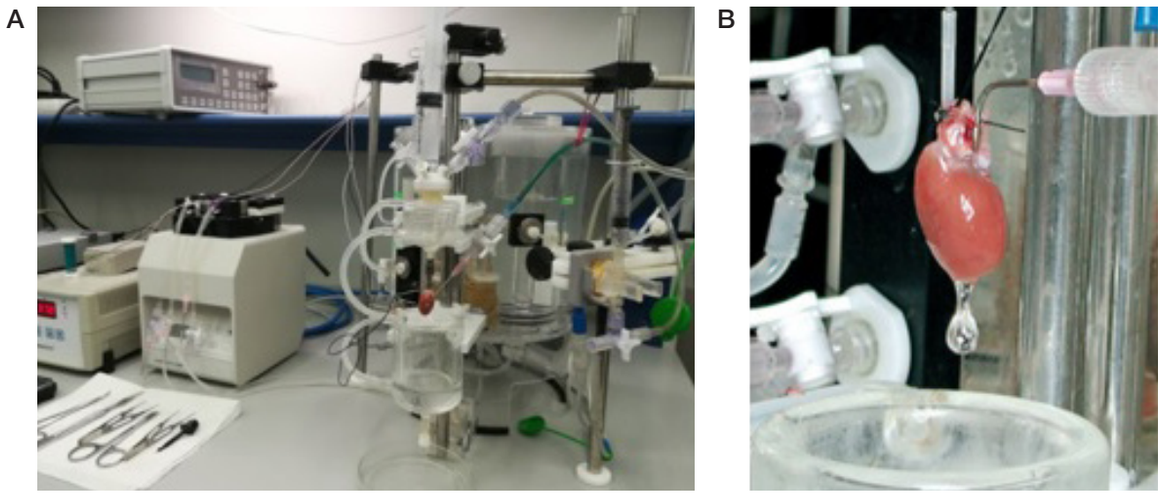

Fig. 2. Installation of an isolated heart according to Langendorff. **A**. General view. **B**. Cannulated heart

to 2.3 ml. For artificial circulation in the pulmonary circuit, we used Krebs-Henseleit solution of the following composition (in mM): NaCl (118.99); KCl (4.69); $NaHCO_3$ (25.00); KH_2PO_4 (1.18); $MgSO_4 \times 7H_2O$ (1.17); $CaCl_2 \times 2H_2O$ (2.5); EDTA (0.03); glucose (5.5). The prepared solution was aerated with a gas mixture containing 5% CO_2 and 95% O_2. In the experimental group, we added the biologically active component to the carbogen-enriched Krebs-Henseleit solution at a concentration of 1×10^{-6} M. The flow rate of the perfusate reached 1.5 ml/min. The duration of the perfusion was 1.5 h; at the end of the experiment, the heart-lung complex was weighed again. In the given experimental conditions, development of the edema is caused by a drop in the perfusate's oncotic pressure: liquid from pulmonary circulation diffuses into the interstitium of the alveoli.

The registered parameters were perfusion pressure in the pulmonary circulation, lung mass and intratracheal pressure.

Isolated heart model

For the isolated heart model experiment, we euthanized the animals by cervical dislocation. Bilateral transabdominal thoracotomy allowed wide access to the cavity. The heart, once exposed, was taken by the base with thumb and forefinger of the left hand, carefully pulled ventrally and downward, then the great vessels were cut with scissors. Immediately after the heart was removed from the chest cavity, it was placed in Krebs-Henseleit saline solution of the following composition (in mM): NaCl (118.99); KCl (4.69); $NaHCO_3$ (25); KH_2PO_4 (1.18); $MgSO_4 \times 7H_2O$ (1.17); $CaCl_2 \times 2H_2O$ (2.5); EDTA (0.03); $C_6H_{12}O_6$ (5.5). The aorta was fixed to the cannula of the Langendorff System perfusion rig (Panlab; Spain) with a crocodile clamp and then with ligatures (Fig. 2).

The heart was perfused through a cannula in the aorta, with the perfusate retrogradely delivered to the left ventricle. The perfusate was Krebs–Henseleit solution warmed to 37 °C. To bring its pH to the physiological level (7.4) and to ensure adequate oxygenation of the heart, the solution was continuously saturated with carbogen. The feeding rate of the perfusate was 10 ml per minute per 1 g of wet weight of the heart. The perfusion adequacy control value was pressure (not less than 50 mm Hg) in the "pump-aortic cannula" circuit.

We used a catheter with a polyethylene balloon to measure pressure in the left ventricle. The parameters of cardiac contractile activity were recorded with the PowerLab Data acquisition system 8/30 (ADInstruments; USA) and subsequently processed in the LabChartProUpgrade 7.0 software (ADInstruments; USA).

The most significant recorded indicators were pulse pressure (PP), heart rate (HR), end-diastolic pressure (EDP), which describes the ability of the myocardium to support cardiac output and create the necessary pressure in the vascular system. Additionally, we calculated the first time derivative of pressure (+dP/dt and –dP/dt), which reflects the rate of pressure change in the left ventricle. The dynamics of +dP/dt and –dP/dt reflects functional state of the ventricles: energy metabolism, permeability of cell membrane ion channels and sarcoplasmic reticulum.

After the stabilization period (30 minutes) was over, we added the active component to the perfusate at the concentration of 1×10^{-6} M. The exposure time was 10 minutes. It was determined by the rate of development of vascular reactions to the vasoactive substance.

To assess the resistance of the isolated myocardium to ischemia/reperfusion, we induced total ischemia by stopping the perfusion for 30 minutes at 37 °C, and then started reperfusion, which lasted for 30 minutes.

Analyzing the results, we assessed the dynamics of functional indicators of isolated heart and lungs, compared them to the background and control values. Statistical processing was performed using GraphPad Prism 5.04 (GraphPad Software Inc; USA). To compare the results with normal distribution of the data, we applied Student's t test; when the distribution was different from normal, we applied the paired samples Wilcoxon test; Mann–Whitney *U* test was

Table 1. Heart-lungs complex weight (M ± SD), *n* = 7

Experimental series	Heart-lungs complex weight, g		Weight gain, %
	at the beginning of the experiment	at the end of the experiment	
Control	4.9 ± 0.5	14.9 ± 3.9	300.7 ± 66.4
Experimental (1 ± 10^{-6} M)	4.9 ± 0.7	10.1 ± 2.3	205.7 ± 42.8*

Note: * — statistically significant differences with the control series of experiments.

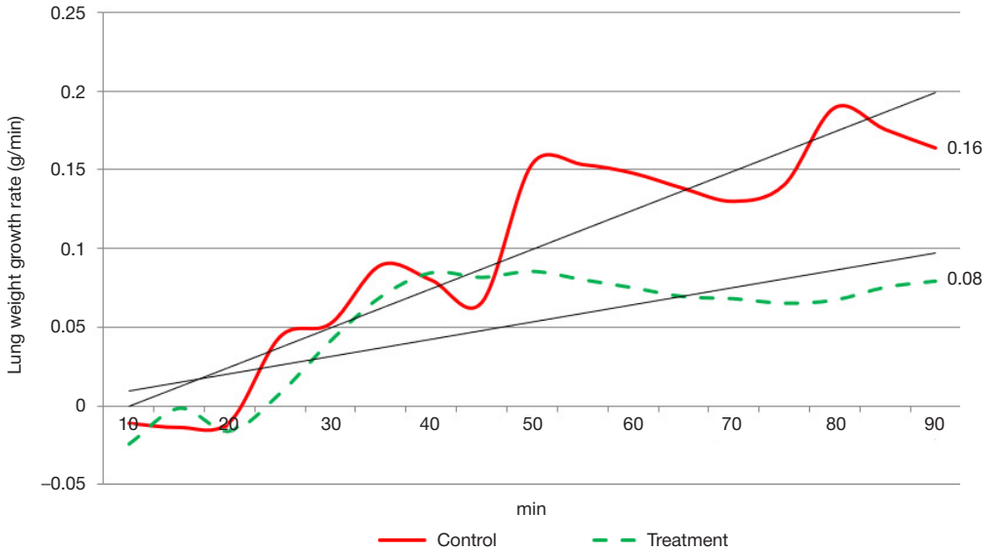

Fig. 3. Lung weight growth rate, treatment and control groups

used to assess intergroup differences. The differences were considered significant at $p \leq 0.05$.

The selected experimental COVID-19 pathogenetic therapy and complications prevention agent was APC (FSUE "NII GPECH" FMBA; Russia) made from thermally treated hydrolyzate of milk protein and enriched with proline and alanine, including linear and cyclic peptides. The active components of the complex improve tissue blood supply by stimulating endothelial nitric oxide synthase. This effect shapes the APC's cardioprotective properties [10]. There seems to be promise in using APC as a stroke prevention agent, part of the ischemic brain damage therapy, as well as an agent alleviating pulmonary edema by decreasing vascular resistance in the pulmonary circulation [7].

RESULTS

The study showed that 1.5 hours of perfusion increased the weight of the heart-lungs complex in both groups, but in the experimental group, this increase was significantly smaller ($p < 0.05$) than in the control group (Table 1).

Starting from the 45–50th minute of exposure, the heart-lungs complex weight growth accelerates significantly in the control group, whereas in the experimental group its rate remains the same (Fig. 3).

As for the perfusion pressure in the pulmonary circulation vessels, it did not differ significantly between groups (Fig. 4), which suggests that APC has little effect on their resistance.

Experimentally, we identified that the resistance of airways of the isolated lungs tends to decrease when the perfusate is supplemented with APC components, which may indicate that pulmonary edema is less severe in the experimental group (Fig. 5).

The heart model isolated in the Langendorff system allowed establishing that the active components of the APC at the concentration of 1×10^{-6} M, which corresponds to the 50 mg/kg dose when administered intragastrically, do not significantly affect functional parameters of an intact rat heart but promote an end-diastolic pressure drop ($p < 0.05$) under ischemia-reperfusion (Fig. 6) and support the rate at which the myocardium contracts and relaxes during the cardiac cycle (Table 2).

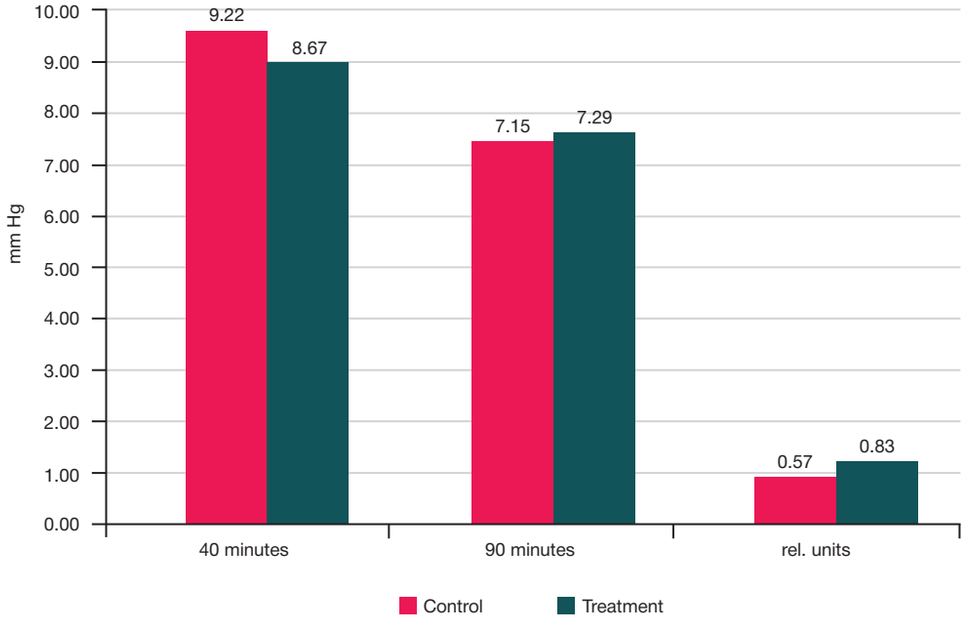

Fig. 4. Isolated lung perfusion pressure dynamics. The ratio of initial and final perfusion pressure is given in relative units

Fig. 5. Isolated lung intratracheal pressure dynamics

Administration of the nitric oxide synthase blocker L-NAME led to a significant end-diastolic pressure growth, which signals of poorer myocardial relaxation and diastolic filling. These changes were also accompanied by a decrease in pulse pressure, which jointly contributed to deterioration of the heart's pumping function.

DISCUSSION

Active components of the studied APC effectively regulate cellular energy metabolism, promote preferential utilization of the more energy-intensive long-chain fatty acids, which supports energy homeostasis, especially in conditions of energy deficiency. Regulation of the transcriptional activity of PPARδ boosts body's endurance, improves blood supply to the tissues and accelerates lipolysis [11]. The APC promotes glucose uptake by direct translocation of GLUT transporters onto plasma membrane, which makes it a promising agent for mitochondrial dysfunction cases [12]. Endothelial nitric oxide synthase is one of the APC's targets, which shapes its cardioprotective properties and substantiates the use of APC as a heart failure prevention agent [13]. In addition, APC helps inhibit secretion of the pro-inflammatory cytokines and has a moderate antimicrobial effect.

According to the data revealed by the study, administration of APC helps maintain the lung mass stable through the

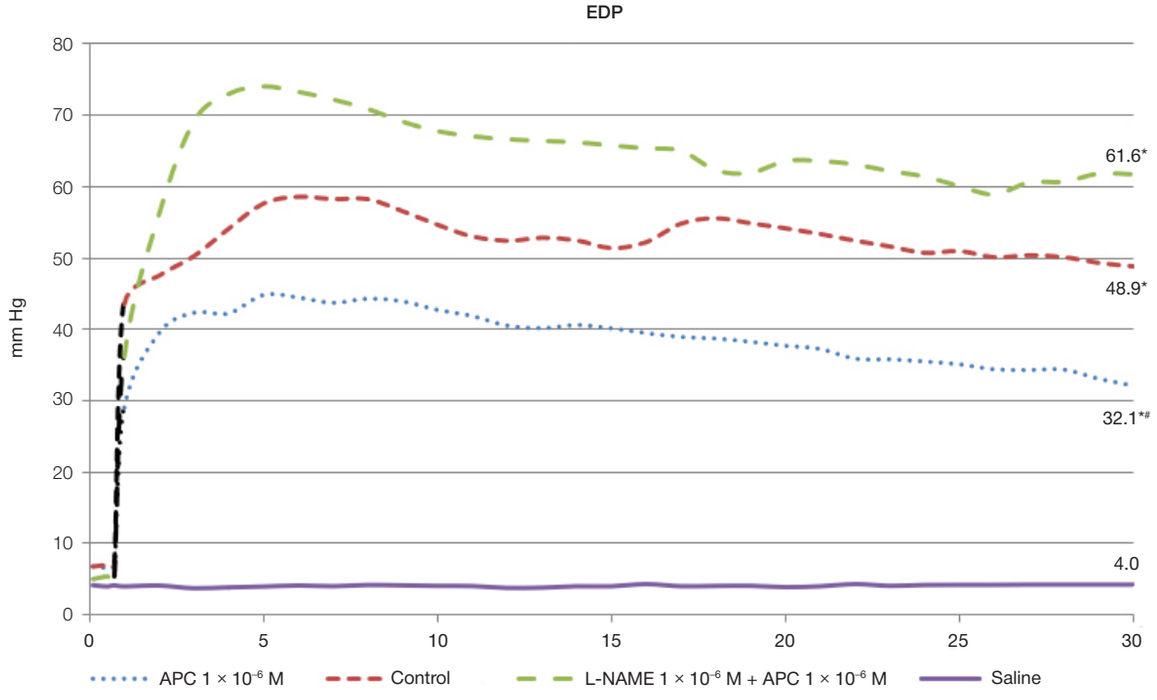

Fig. 6. End diastolic pressure dynamics, isolated heart during reperfusion after 30 minutes of total normothermic (37 °C) ischemia. APC administered. * — the differences are statistically significant in comparison with the intact organ; # — the differences are statistically significant in comparison with the "reperfusion (control)" group ($p < 0.05$)

Table 2. Indicators of myocardial contractility with APC administered. The data provided are relative to the background, in % (M ± SE); for the EDP the data is absolute, $n = 7$

Perfusate	Heart rate	PP	+ dP / dt	– dP / dt	EDP (mm Hg)
Saline, 30 min	91.6 ± 3.4	101.5 ± 3.4	103.1 ± 2.1	96.7 ± 3.1	4.0 ± 1.6
APC1 × 10^{-6} M	101.0 ± 1.8	99.0 ± 2.1	99.0 ± 0.9	99.5 ± 0.7	3.9 ± 2.4
Reperfusion, control	91.1 ± 7.1	53.7 ± 7.6*	58.7 ± 7.3*	70.3 ± 7.7*	48.9 ± 4.9*
Reperfusion APC 1 × 10^{-6} M	95.0 ± 4.5	64.0 ± 12.5*	80.9 ± 10.6*	95.6 ± 9.2#	32.1 ± 5.7*#
Reperfusion L-NAME 1 × 10^{-6} M + APC 1 × 10^{-6} M	95.5 ± 1.9	41.2 ± 11.5*	54.9 ± 9.5*	73.2 ± 13.9*	61.6 ± 6.7*

Note: * — the differences are statistically significant in comparison with the intact organ; # — the differences are statistically significant in comparison with the "reperfusion (control)" group ($p < 0.05$)

experiment, possibly due to the decreasing permeability of the blood-air barrier. As a result of 1.5 hours of perfusion, the weight of the isolated heart-lungs complex increased in both groups, but in the experimental group this increase was 1.5 times smaller than in the control group ($p = 0.0158$). The difference, detectable from the 40th minute of perfusion, is obviously caused by the greater excursion of the lungs triggered by the increasing tidal volume (up to the recommended values, as per the methodology) [14]. This growth of the lung volume can raise pressure gradient between the perfused vessels of pulmonary circulation and the alveolar space, which facilitates release of fluid into the interstitium of the alveoli and promotes edema development [15].

The experiments on an intact isolated heart showed that the studied APC produces no cardiotropic effects, as evidenced by the unchanging values of the myocardial functional activity indicators. Indirectly, ischemia breaks the heart's energy balance, which translates into deteriorating diastolic function, pulse pressure and cardiac output. Introduction of the APC into the isolated heart's perfusate 10 minutes before onset of the total normothermic ischemia slowed down the growth of end-diastolic pressure registered, which reflects the ability of the myocardium to relax, as well as to allow a more complete recovery of the pulse pressure, maximum rate of contraction and relaxation of the left ventricle, with the said growth slower than that registered in the control group ($p < 0.05$) during reperfusion. Thus, the studied APC, when administered to treat ischemic conditions, can reinforce the heart's resistance to insufficiencies of oxygen supply, energy substrates, and increase the stability of the cell membranes of cardiomyocytes under reperfusion [16].

The experiments with L-NAME nitric oxide synthase blocker revealed that the APC's cardioprotective action is endothelium-dependent and results from the activation of NO synthase.

Thus, an isolated heart-lungs complex allows simulating development of the pulmonary edema peculiar to COVID-19, which can be used to assess the efficacy of therapeutic and complications prevention agents. Pharmacological action of the active components of APC offers a potential therapeutic way to reduce the magnitude of ischemic damage to the myocardium, preserve energy reserves, restore metabolism and contractile function [17].

CONCLUSIONS

The study revealed that administration of the APC to an isolated heart-lungs complex significantly reduces the pulmonary edema development rate, with the possible reasons therefor being deterioration of permeability of the blood-air barrier (for perfusate) and the intratracheal pressure's downtrend. The APC offers a cardioprotective effect, helps maintain the effectiveness of myocardial relaxation in diastole and, consequently, reduce the end-diastolic pressure. The use of isolated organs (heart-lungs complex) allows adequate assessment of the parameters of functional activity of vital organs when simulating processes close to the physiological norm, as well pathological conditions, such as, in particular, pulmonary edema and myocardial hypoxia. The method is highly sensitive and enables evaluation of reactivity of the systems exposed to biologically active substances in a wide range of concentrations, as well as identification of the functional change compensation capabilities.

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EFFECT OF INTERMITTENT HYPOXIC TRAINING ON ORTHOSTATIC TOLERANCE IN HUMANS BEFORE AND AFTER SIMULATED MICROGRAVITY

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Reduced orthostatic tolerance (OT) is a serious concern facing space medicine. This work sought to evaluate the effects of intermittent hypoxic training (IHT) on OT in humans before and after 3 days of head-down bed rest (HDBR) used to model microgravity. The study was carried out in 16 male volunteers aged 18 to 40 years and included 2 series of experiments with 11-day and 21-day IHT administered on a daily basis. During the first IHT session, the concentration of oxygen in the inspired gas mixture was 10%; for other sessions it was adjusted to 9%. OT was assessed by a 20-minute-long orthostatic tilt test (OTT) conducted before and after HDBR. Before HDBR, orthostatic intolerance was observed in 3 participants, while after HDBR, it was observed in 9 of 16 volunteers ($p < 0.05$). During OTT conducted after HDBR, the heart rate (HR) exceeded control values by 26.8% ($p < 0.01$). Preexposure to any of the applied IHT regimens led to a reduction in the number of volunteers with orthostatic intolerance. After the 11-day IHT program, there was a less pronounced increase in HR during OTT before HDBR; with the extended IHT regimen, less pronounced changes were observed for HR, systolic, diastolic and mean blood pressure (BP). The increase in HR during OTT after HDBR was significantly lower in the group that had completed the 11-day IHT program, while BP remained stable. The changes in HR and systolic BP were less pronounced in the group that had completed the 21-day IHT program than in the control group ($p < 0.05$). Thus, IHT reduced the risk of orthostatic disorders and mitigated changes in cardiovascular parameters during the orthostatic test.

Keywords: intermittent hypoxic training, orthostatic tolerance, head-down bed rest, blood pressure, heart rate

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Author contribution: Katuntsev VP conceived and designed the study, wrote the manuscript; Sukhostavtseva TV collected and analyzed the obtained data, performed statistical analysis and edited the manuscript; Kotov AN collected and analyzed the obtained data and performed statistical analysis; Baranov MV collected and analyzed the obtained data and edited the manuscript.

Compliance with ethical standards: the study was approved by the Ethics Committee of Federal Research Clinical Center of FMBA (Protocol № 1 dated February 7, 2019) and conformed with the principles of biomedical ethics laid out in the Declaration of Helsinki (the 1964 version and subsequent updates); voluntary informed consent was obtained from each study participant.

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ВЛИЯНИЕ ГИПОКСИЧЕСКИХ ТРЕНИРОВОК НА ОРТОСТАТИЧЕСКУЮ УСТОЙЧИВОСТЬ ЧЕЛОВЕКА ДО И ПОСЛЕ МОДЕЛИРОВАННОЙ МИКРОГРАВИТАЦИИ

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Снижение ортостатической устойчивости (ОУ) является актуальной проблемой космической медицины. Целью работы было оценить влияние интервальных гипоксических тренировок (ИГТ) на ОУ человека до и после воздействия трехсуточной антиортостатической гипокинезии (АНОГ) как модели микрогравитации. При участии 16 мужчин-добровольцев в возрасте 18–40 лет проведены две серии исследований с 11- и 21-суточным курсом ежедневных ИГТ. В первой ИГТ концентрация кислорода во вдыхаемой газовой смеси составляла 10%, во всех последующих — 9%. Оценку ОУ выполняли до и после АНОГ проведением 20-минутной ортопробы (ОП). Развитие ортостатической неустойчивости до АНОГ наблюдали у трех, а после АНОГ у девяти из 16 обследуемых ($p < 0,05$). Во время ОП после АНОГ среднее значение частоты сердечных сокращений (ЧСС) превышало контрольные значения на 26,8% ($p < 0,01$). После 11- и 21-суточных ИГТ отмечена тенденция к снижению числа случаев с развитием ортостатической неустойчивости. По сравнению с контролем при ОП до АНОГ после 11-суточного курса ИГТ наблюдали менее выраженный прирост ЧСС, а при увеличении курса ИГТ до 21 суток — менее выраженные реакции со стороны ЧСС, систолического, диастолического и среднего артериального давления (АД). При ОП после АНОГ в серии с 11-суточным курсом ИГТ имело место достоверно меньшее увеличение ЧСС при стабильном уровне АД. В серии с 21-суточным курсом ИГТ наблюдали меньшие сдвиги ЧСС и систолического АД ($p < 0,05$). Таким образом, проведение ИГТ приводило к уменьшению риска ортостатических нарушений и менее выраженным сдвигам показателей сердечно-сосудистой системы во время постуральных воздействий.

Ключевые слова: интервальные гипоксические тренировки, ортостатическая устойчивость, антиортостатическая гипокинезия, артериальное давление, частота сердечных сокращений

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Exposure to a natural or modelled microgravity environment leads to the deconditioning of the physiological systems involved in maintaining the upright posture under Earth's gravity. Diminished orthostatic tolerance (OT) is a serious symptom of deconditioning that was recognized in the early days of manned space missions [1, 2]. After short-duration Space Shuttle flights, about 20% of astronauts were unable to complete a 10-minute orthostatic tilt test (OTT) due to a progressive blood pressure fall and presyncope [3]. Even more American astronauts developed orthostatic intolerance after long-duration missions aboard Mir [3] and the International Space Station (ISS). Besides, ISS astronauts took longer to recover than Space Shuttle crews [4].

Countermeasures against the adverse effects of microgravity on the human body during orbital flights are complex, time-consuming and include daily exercise for about 2.5 hours [5]. However, they cannot completely avert the development of orthostatic intolerance in the early postflight period [4, 6]. The first Russian experimental studies investigating the effects of the modeled lunar gravity field on human physiology [7] underscore the significance of this yet unsolved problem for future space missions [8].

Manned space missions to the Moon and beyond to Mars will require more effective and less time-consuming countermeasures enhanced by cutting-edge technologies against the deconditioning effects of micro- and hypogravity on gravity-dependent body systems. Creating artificial gravity on board of a spacecraft is the most radical solution to counter microgravity [9]; in turn, methods for targeted physiological action [10], including adaptation to hypoxic hypoxia [11], might reinforce the effect.

Today, adaptation to hypoxic hypoxia through normobaric or hypobaric intermittent hypoxic training (IHT) is widely used in clinical, sports, aviation and space medicine as a non-drug therapy for restoring body function, improving physical performance and resisting occupational stress [12, 13]. According to some publications, IHT can reduce the intensity of hemodynamic changes during orthostatic tests [14, 15]. It is reported that a 14-day-long exposure to a hypoxic environment reduces orthostatic hypotension and increases orthostatic tolerance in rats kept in the antiorthostatic position (modeled microgravity) for 2 weeks [16]. The findings of the cited study inspired us to carry out an experiment on human subjects in the attempt to investigate the effects of IHT on OT before and after a 3-day exposure to modeled microgravity.

METHODS

The study was carried out in 16 healthy, non-smoking male subjects aged 18 to 40 years (the mean age was 26.4 ± 1.5 years; the mean body weight, 76.8 ± 2.6 kg; height, 177 ± 1.9 cm) and not involved in professional sports. The following inclusion criteria were applied: approval by the medical board and informed consent to participate. Two days before the experiment, the subjects were accommodated in an inpatient unit for adaptation. During the adaptation period, their condition was closely monitored by the medical personnel. Physical loads were banned. Meals were provided 4 times a day. Sleep time was from 23:00 to 8:00. Microgravity was simulated by 3 days of –6° head-down tilt bed rest (HDBR) [17].

IHT sessions were conducted using a Bio-Nova-204 system for hypoxic therapy (Bio-Nova; Russia). The hypoxic gas mixture was delivered to the seated participants through a mask pressed tightly against the face, in a well-ventilated room for physiological tests involving humans. IHT sessions were held daily and lasted 60 min each. Each session consisted of 6 cycles: 5-minute periods of breathing the hypoxic gas mixture followed by 5 minutes of breathing ambient air. During the first IHT session, the concentration of oxygen in the inspired gas mixture (FIO_2) was 10%. Starting from the 2nd session, FIO_2 was adjusted to 9%. During IHT, the condition of the participants was closely monitored; oxygen saturation (SpO_2), heart rate (HR), systolic and diastolic pressures (BP) were taken every 3 minutes.

OT was assessed a day before HDBR and immediately after 3 days of HDBR on a tilt table by transferring the subjects to a vertical position at an angle of + 70 ° for the maximum of 20 min. Before, during (every 2 minutes) and after the end of OTT, HR, systolic and diastolic BP were measured. Subjective and objective indicators of health status were evaluated. Prior to OTT, baseline physiological parameters were recorded in the supine position (before hypokinesia) and in the HDBR position (after hypokinesia).

Physiological parameters were measured using a PVM-2703 bedside monitor (Nihon Kohden Corporation; Japan) fitted with a pulse oximeter and channels for measuring BP and ECG. Mean BP was computed as the sum of diastolic BP and 1/3 of pulse pressure. OTT was terminated if the tested participant had a progressive BP decrease, bradycardia, nausea, excessive sweating, blurred vision and other signs of imminent syncope. Two different IHT regimens were used. In the first part of the experiment, IHT duration was 11 days; in the second part, IHT was extended to 21 days. The number of the participants involved was 6 and 11, respectively.

Statistical analysis was carried out in Microsoft Excel ver. 2016 (16.0.5071.1000; Microsoft Corporation; USA). Significance of differences was assessed using the nonparametric Wilcoxon signed-rank test, the Mann–Whitney *U* test and Fisher's — criterion. Differences were considered significant at $p < 0.05$. The table and figures below show the mean values of the studied parameters and the mean error (M ± m).

RESULTS

IHT tolerance by subjects

During hypoxic gas breathing, the subjects did not feel any discomfort or had any health complaints. SpO_2 was falling from 97.0 ± 0.5% to 77.6 ± 2.6%; HR was increasing from 71.7 ± 4.0 min^{-1} to 89.0 ± 4.3 min^{-1} ($p < 0.05$). BP did not change significantly. When the participants were breathing ambient air, their SpO_2 and HR were recovering, reaching the initial values by the beginning of the next IHT cycle.

Table. Effects of 3-day HDBR on orthostatic tolerance in subjects

Orthostatic tilt test parameters	Before HDBR	After HDBR
Number of completed OTT/total number of OTT	13/16	7/16*
Average test duration, min	18.6 ± 0.8	13.8 ± 1.6*
Average time to presyncope, min	12.7 ± 1.6	9.0 ± 1.4*

Note: OTT — orthostatic tilt test; * — $p < 0.05$.

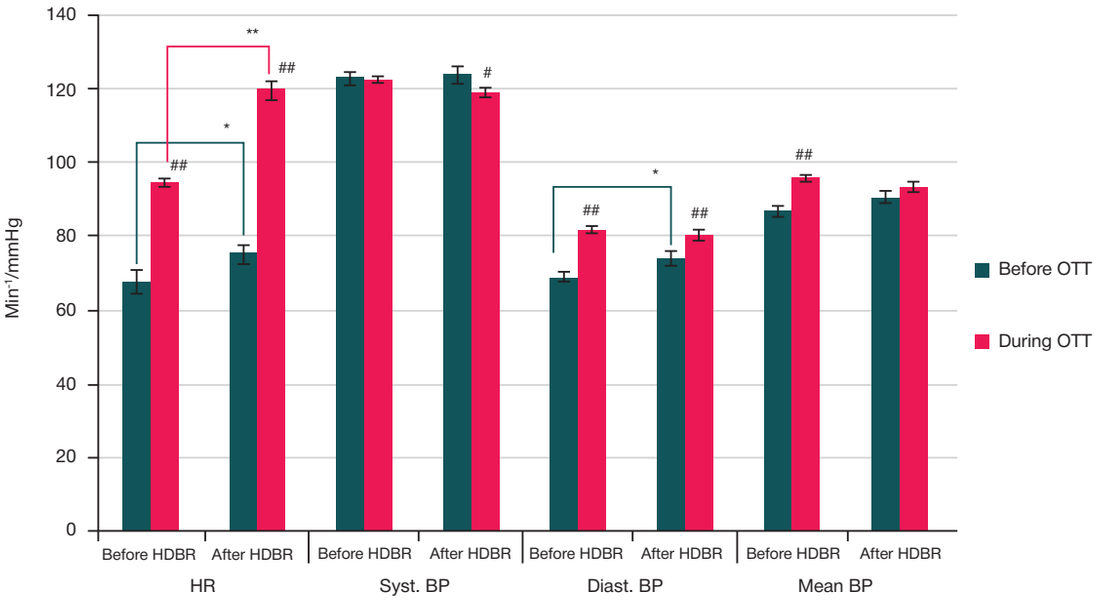

Fig. 1. Effects of 3-day HDBR on cardiovascular responses to the orthostatic test. # ($p < 0.05$) and ## ($p < 0.01$) designate differences between the data obtained during OTT and the data obtained from supine subjects before OTT; * ($p < 0.05$) and ** ($p < 0.01$) designate differences before and after HDBR

Effects of 3-day HDBR on orthostatic tolerance

The Table below shows the results of OTT before and after 3 days of HDBR. After HDBR, the number of successfully completed OTTs dropped from 13 to 7, whereas the number of OTTs terminated due to the symptoms of presyncope increased threefold, from 3 to 9 ($p < 0.05$). For the group, the average time of OTT after HDBR significantly decreased by 4.8 min ($p < 0.05$) in comparison with the control.

In addition to the increased number of presyncopies, time from tilting the subjects upward to the onset of presyncopal symptoms also tended to decrease by 3.7 min.

Following 3 days of HDBR, the mean HR during OTT exceeded the control values by 26.8% and was 119.8 ± 2.6 min^{-1} vs. 94.6 ± 0.9 min^{-1} before HDBR ($p < 0.01$; Fig. 1). Moreover, the post-HBDR HR was significantly higher in the experimental group than in the controls throughout the test (Fig. 2). The significant increase in HR was accompanied by a slight (about 5%) yet reliable mean systolic BP fall from 123.8 ± 2.2 to 118.8 ± 1.3 mmHg and an elevation of diastolic BP, which was less pronounced in the experimental group: 8.9% (from 73.9 ± 1.6 to 80.5 ± 1.2 mmHg) vs. 18.5% in the control group (from 69.2 ± 1.4 to 82 ± 0.6 mmHg). There was no reliable increase in the mean BP (see Fig. 1). Of note, the absolute values of HR and diastolic BP measured in the supine position before the initial OTT were 11% and 6.8% lower, respectively, than the absolute values of HR and diastolic BP measured in the antiorthostatic position before the post-HDBR tilt test ($p < 0.05$).

Effects of 11-day IHT on orthostatic tolerance

In the first part of the experiment, OTTs (before and after HDBR) were carried out on 6 participants. Later, one of them decided to drop out. Consequently, the effects of IHT on orthostatic tolerance before and after HDBR were investigated in a group of 5 individuals, and the data on the dropout was not included in the analysis.

Before HDBR, the tilt test was completed by 4 (80%) of 5 participants; after IHT, 5 of them (100%) were able to pass the test. Initially, of 5 OTTs performed after HDBR, 3 (60%) were

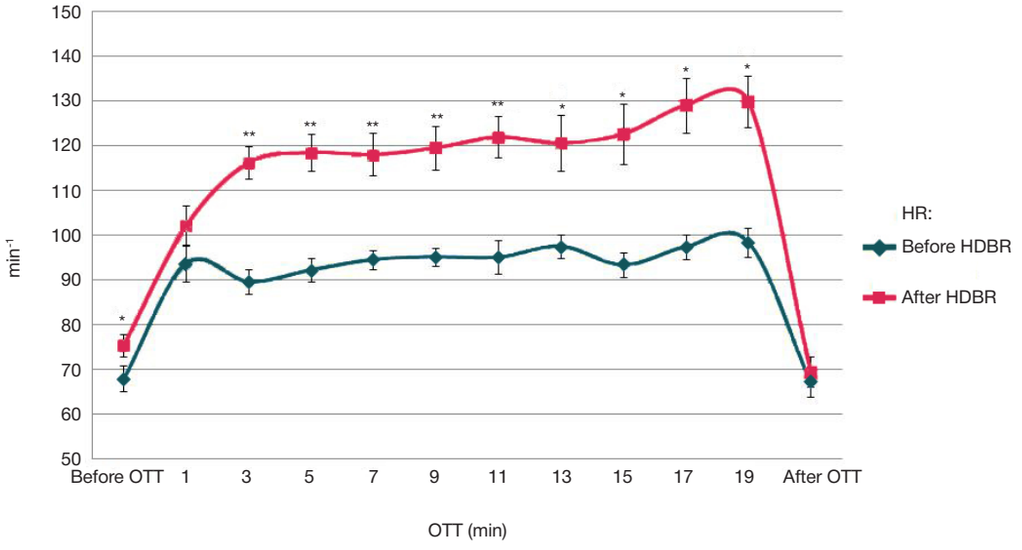

Fig. 2. HR dynamics during the orthostatic test before and after 3 days of HDBR.* ($p < 0.05$) and ** ($p < 0.01$) designate differences before and after HDBR

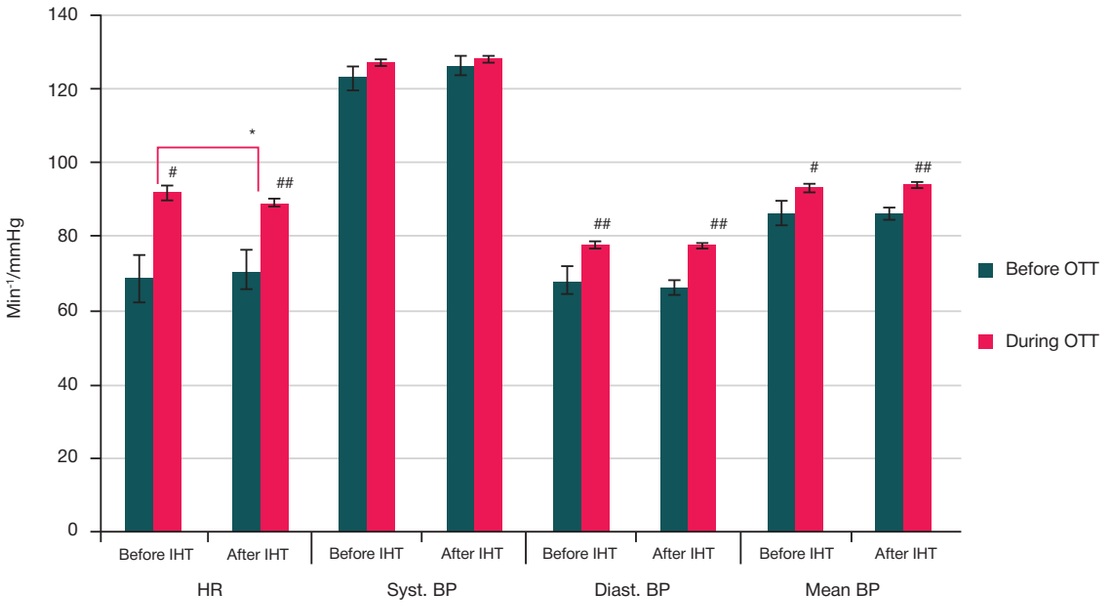

Fig. 3. Effects of 11-day IHT on cardiovascular responses to the orthostatic test before HDBR. # ($p < 0.05$) and ## ($p < 0.01$) designate differences between the data obtained during OTT and the data obtained from supine subjects before OTT; * ($p < 0.05$) marks differences before and after IHT

terminated because the participants became presyncopal. However, IHT presyncopal symptoms were observed in only one (20%) of 5 participants. The mean OTT duration tended to increase from 13.4 ± 3.5 min to 18.6 ±1.6 min.

The effects of 11-day IHT on the cardiovascular system undergoing orthostatic exposure are shown in Fig. 3. IHT before HDBR resulted in a less pronounced (3%) increase in HR ($p < 0.05$) in comparison with no IHT. Interestingly, the increase in HR during OTT after HDBR was much less pronounced (16.1%; $p < 0.05$) in the participants who had completed the IHT program than in the control group (Fig. 4). Other IHT effects included a stable systolic BP and a higher mean BP (7.2%; $p < 0.05$).

Effects of 21-day IHT on orthostatic tolerance

Of 10 participants included in the second part of the experiment, 8 (80%) individuals were able to successfully complete pre-IHT OTT before HDBR, whereas after IHT 9 (90%) subjects were able to pass the test. In 3 cases (2 before IHT and 1 after IHT), OTT was terminated because the participants developed the symptoms of presyncope. A slight (4.9%) increase in mean orthostatic tolerance (18.2 ± 1.2 vs. 19.1 ± 0.9 min) was observed in the participants who had undergone the IHT program, as compared with the control group.

Prior to IHT, 4 (40%) of 10 participants were able to complete OTT after HDBR; their number increased to 6 (60%) after IHT. In 6 cases before IHT and 4 cases after IHT, OTT was terminated because the participants were showing the signs of presyncope. There was a tendency to better tilt test tolerance in the group that had undergone the IHT program: the test duration increased from 13.4 ± 2.1 to 14.7 ± 2.2 min, i.e. by 9.7%, in this group as compared to the control.

The effects of IHT on hemodynamics observed during OTT before HDBR are provided in Fig. 5. In comparison with the control group, HR, diastolic BP and mean BP increased

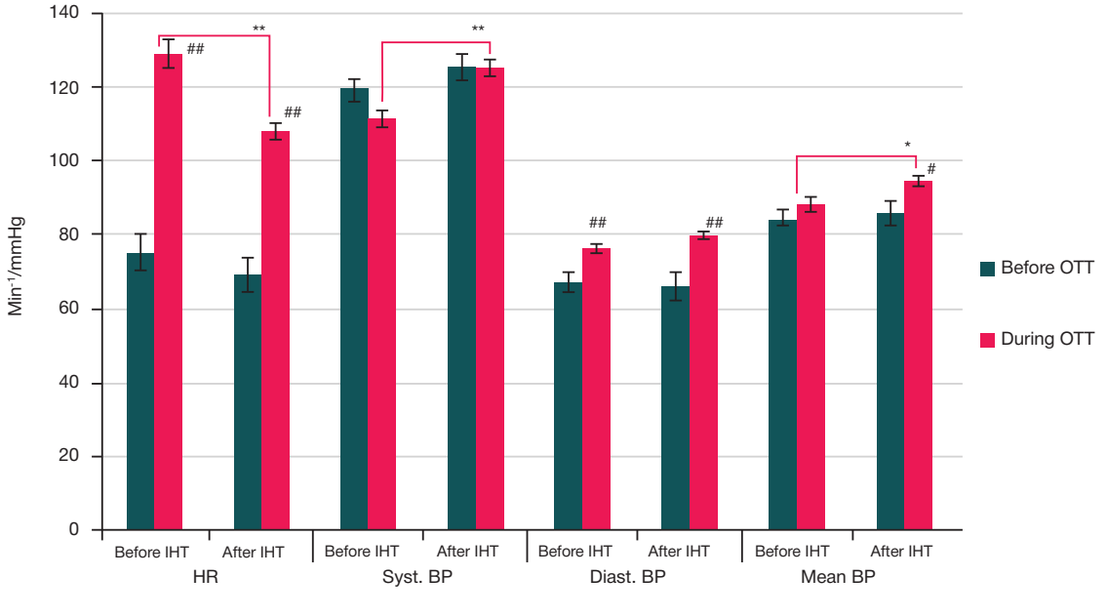

Fig. 4. Effects of 11-day IHT on cardiovascular responses to the orthostatic test after HDBR. # ($p < 0.05$) and ## ($p < 0.01$) designate differences between the data obtained during OTT and the data obtained from supine subjects before OTT; * ($p < 0.05$) and ** ($p < 0.01$) designate differences before and after IHT

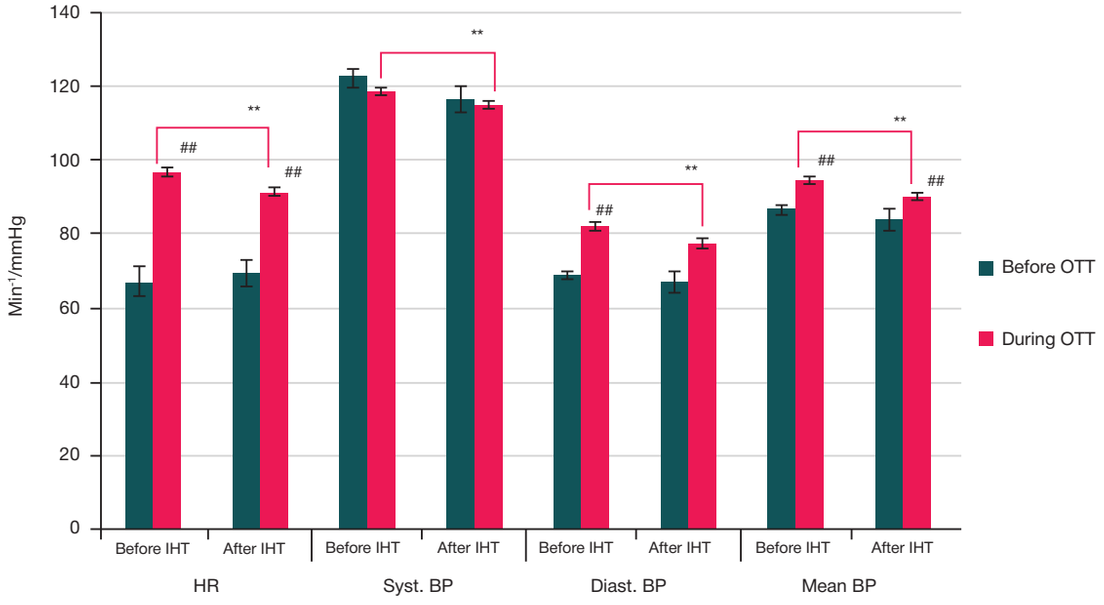

Fig. 5. Effects of 21-day IHT on cardiovascular responses to the orthostatic test before HDBR. # ($p < 0.05$) and ## ($p < 0.01$) designate differences between the data obtained during OTT and before OTT; * ($p < 0.01$) marks differences before and after HDBR

less dramatically during OTT (by 5.4%, 6.3% and 5.1%, respectively; $p < 0.01$) in the participants who had completed the IHT program. Systolic BP did not change significantly during OTT but was 3.3% lower than before IHT ($p < 0.01$).

During the post-HDBR tilt test (Fig. 6) performed after IHT, an increase in HR was less pronounced (4.6%) and BP values were lower (5.8%) ($p < 0.05$). Before OTT, HR, diastolic BP and mean BP were 14.5%, 5.1% and 4.3% lower in the participants who had completed the IHT program than in the control group ($p < 0.05$).

DISCUSSION

The study demonstrates that 3 days of HDBR reduces OT in human subjects. After HDBR, significantly fewer participants could complete the test due to the symptoms of presyncope and the trending early onset of such symptoms in the vertical position. After 3 days of HDBR, orthostatic intolerance was observed in 9 (56.3%) of the total 16 participants. Our findings support the data generated by other studies. It is reported that after 4 days of HDBR, as many as 5 (63%) of 8 subjects were unable to finish the orthostatic test [18, 19]. There is evidence that orthostatic intolerance can develop after shorter exposures to HDBR. For example, 6 (75%) of 8 participants became presyncopal during OTT after only 4 h of HDBR [20].

Differences in the estimated frequency of orthostatic intolerance after HDBR might largely be due to the employment of different methods for OTT, different tilt table angles (60 to 80°), OTT duration (10–20 to 60 min), application of negative pressure to the lower body after OTT, nonuniform criteria for assessing OT (based on test duration or the onset of presyncope), individual physiological response to OTT [21, 22].

The key role in maintaining systemic BP and cerebral circulation during orthostatic exposure is attributed to the cardiovascular system [23]. In our study, the hemodynamic response to orthostatic exposure was characterized by

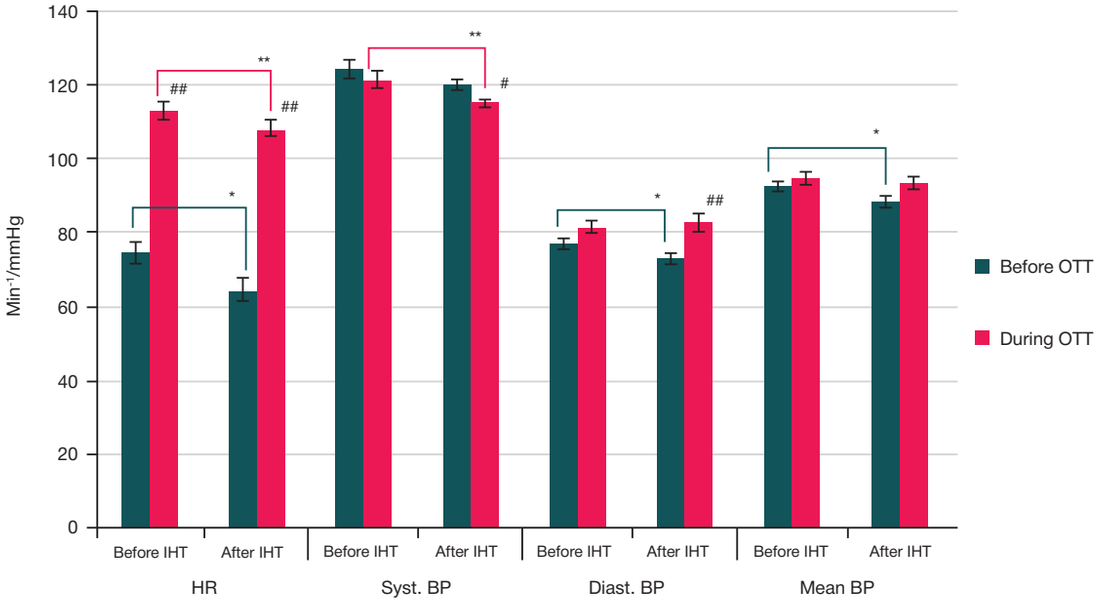

Fig. 6. Effects of 21-day IHT on cardiovascular responses to orthostatic test after HDBR. # ($p < 0.05$) and ## ($p < 0.01$) designate differences between the data obtained during OTT and before OTT; * ($p < 0.05$) and ** ($p < 0.01$) designate differences before and after IHT

pronounced tachycardia and low systolic BP after HDBR. These findings are consistent with the results of space flight studies [24] and studies of antiorthostatic hypokinesia [25].

Pronounced tachycardia observed during OTT after HDBR should be considered a symptom of cardiovascular deconditioning caused by hypokinesia. It is known that –6° HDBR leads to blood/fluid redistribution toward the skull and increases the blood volume in the thoracic compartment [26]. Increased venous return to the right atrium triggers secretion of the atrial natriuretic peptide [27]. This results in reduced water reabsorption, diuresis, increased natriuresis and, eventually, decreased plasma volume. After 2 days of HDBR, the central blood volume drops by approximately 11% [28], and the plasma volume decreases by 6.1% [29]. It normally takes 2 to 4 days for the cardiovascular and related systems to adapt to HDBR; the adaptive state is characterized by slower HR and slightly lower BP [30]. Higher HR and diastolic BP in the antiorthostatic position before OTT after 3 days of HDBR vs. HR and BP in the horizontal position before the initial OTT observed in our study suggest that the body was still adjusting its water-electrolyte balance to the new environment.

In the setting of moderate hypovolemia that develops after 1 week of a spaceflight/ HDBR, the left ventricular end-diastolic volume, the stroke volume and the cardiac size diminish [31]. Apart from the small stroke volume, increased venous distension in the lower limbs, which often develops during HDBR and spaceflights, is also a precipitating factor for orthostatic disorders: orthostatic exposure increases blood flow to leg veins and makes it difficult for the body to maintain adequate cardiac output in the vertical position [32].

The baroreflex mechanism relying on the receptors of carotid sinuses and the aortic arch is the crucial component of neural circulatory control. The baroreflex regulation of BP is largely implemented through the modulation of HR and the vasomotor activity of the sympathetic nervous system (SNS) [33]. A positive correlation has been established between the level of vasomotor SNS activity and total vascular resistance in young men [34]; unlike changes in the central hemodynamics and HR reported by another study [35], vascular resistance turned out to be critical in maintaining BP in astronauts during OTT after short-duration (9–14 days) space missions. These results are well correlated with the data generated by another study [36]. According to the publication, preexposure prophylaxis with midodrine, which is known to enhance vasoconstriction, prevented syncope due to orthostatic exposure in all of 5 study participants following their return to Earth. It is reported that the baroreflex control of vasomotor SNS activity is weakened during HDBR and the subsequent OTT [21]. Today, it is believed that decreased baroreflex sensitivity is one of the principal causes of poor orthostatic tolerance in the setting of hypokinesia and microgravity [21, 25, 30].

Performing IHT reduces the risk of orthostatic disorders. This can be inferred from the less pronounced changes in cardiovascular parameters during orthostatic exposure and fewer cases of presyncopal symptoms before and after HDBR. The increase in HR during OTT before HDBR was smaller in the group that had completed the 11-day IHT program than in the control group. The extended 21-day IHT program led to less pronounced changes in HR, systolic, diastolic and mean BP. The increase in HR during OTT after HDBR was significantly lower in the participants who had completed the 11-day IHT program; at the same time, systolic BP was stable. Both HR and systolic BP were lower in the group subjected to the extended IHT regimen. HR, systolic and diastolic BP values after IHT preceding the test were lower than before IHT, suggesting faster adaptation to HDBR.

According to the literature, the beneficial effects of IHT observed in our study might be connected to certain changes in the functional state of the autonomic nervous system and the cardiovascular system occurring during adaptation to repeated hypoxic exposure [11]. The mechanisms of immediate adaptation to hypoxia rely on the sympathetic activation of the compensatory cardiorespiratory response, which aims to reduce arterial hypoxemia and improve oxygen transport to tissues [16]. Repeated exposure to moderate hypoxia and reoxygenation create a structural and functional basis for the mechanisms that underlie long-term adaptation to hypoxia and improve oxygen uptake by mitochondria [37]. The long-term effects of IHT include enhanced performance of the parasympathetic components of circulatory control and higher efficacy of baro-and chemoreceptor-based regulation of heart rhythm and vascular tone [38]. Regional blood flow is redistributed toward the brain and the heart. IHT has been shown to exert a beneficial effect on vascularization and myocardial contractility [39]. This leads us to hypothesize that IHT might have had a cardioprotective effect on our subjects by increasing myocardial capacity and negating the main detrimental effects of orthostasis manifested as a dramatic BP decline.

CONCLUSION

Pronounced tachycardia during OTT after HDBR should be considered a sign of cardiovascular deconditioning due to limited physical activity during hypokinetic periods. Preexposure to IHT ameliorates cardiovascular strain during orthostatic tests before and after 3 days of HDBR. IHT reduces the risk of orthostatic syncope. The mechanisms underlying IHT effects on the functional state and ratio of cardiac to vascular components maintaining circulatory homeostasis during orthostatic exposure require further elucidation.

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AN EXPERIMENT ON BIOLOGICAL OBJECTS: COMPOSITE FACIAL GRAFT CROSS-TRANSPLANTATION

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Facial graft transplantation remains the operation of choice for patients with extensive tissue defects in the maxillofacial region. This study aimed to set up an experiment on biological objects, develop and test a combined facial graft cross-transplantation technique, select the anesthetic aid allowing to reduce the risks of perioperative complications, improve survivability of the subjects by reducing the duration of surgical intervention, develop a postoperative therapy and rehabilitation protocol, assess detection of an acute rejection reaction and develop the immunosuppressive therapy protocol. We conducted three series of facial graft transplantation surgeries on 26 minipigs and tested the typical component combinations and flap designs. At all stages of the experiment, we managed to have the subjects surviving for over 30 days without disrupting their vital functions. The immunosuppression procedure was developed and tested. The chosen technique allows transplanting two grafts within a single surgery on one pair.

Keywords: face transplant, microsurgery, facial flap, composite flap

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Compliance with ethical standards: the living conditions of animals, care and all manipulations they were subjected to meet the experimental model research standards.

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ПЕРЕКРЕСТНАЯ ПЕРЕСАДКА КОМБИНИРОВАННОГО ЛИЦЕВОГО ТРАНСПЛАНТАТА В ЭКСПЕРИМЕНТЕ НА БИООБЪЕКТАХ

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Пересадка лицевого трансплантата остается операцией выбора для пациентов с обширными дефектами тканей челюстно-лицевой области. Целью работы было в эксперименте на биообъектах разработать и апробировать методику перекрестной пересадки комбинированного лицевого трансплантата, подобрать анестезиологическое пособие с целью снижения рисков периоперационных осложнений, улучшения показателей выживаемости особей за счет сокращения длительности хирургического вмешательства и разработать протокол послеоперационной терапии и реабилитации особей, оценки диагностики острой реакции отторжения и отработки иммуносупрессивной терапии. В трех сериях операций по пересадке лицевых трансплантатов на 26 минипигах были апробированы типичные комбинации компонентов и дизайны лоскута. На всех этапах эксперимента команда добилась выживания особей более 30 дней, без нарушения жизненных функций. Отработана схема иммуносупрессии. Выбранная методика позволяет проводить две пересадки за одно хирургическое вмешательство внутри одной пары.

Ключевые слова: трансплантация лица, микрохирургия, лицевой лоскут, композитный трансплантат

Финансирование: согласно гранту ФМБА на выполнение прикладной научно-исследовательской работы по теме «Исследование метаболических, морфометрических и функциональных характеристик тканей и органов после операций в области головы и шеи на основе применения физических и лазерно-конверсионных цифровых технологий» («ЧЛХ-18»).

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Соблюдение этических стандартов: условия содержания животных, уход и все проводимые с ними манипуляции соответствовали стандартам работы с экспериментальными моделями.

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Currently, the main method for reconstruction of extensive head and neck defects is free autograft transplantation [1–3]. However, the loss of such structures as lips, eyelids, nose makes allotransplantation of a composite facial flap the only approach allowing fully-fledged rehabilitation [4–6].

To date, 40 composite facial graft transplantation surgeries have been executed in the world. The first successful operations were performed in 2005 [7], yet this type of surgical intervention remains unique and requires involvement of highly qualified specialists in the preparation, intervention itself, further observation and rehabilitation [8]. The high immunogenicity of the skin, which increases the risks of graft rejection, is still a big problem faced by the teams performing such manipulations. Currently, there is no single approach to the intervention, with a

Fig. 1. Experimental animals in the immediate postoperative period

Fig. 2. Minipigs on the 14th day after the cross-transplantation

number of solutions suggested. Humanity of experiments and preservation of life of experimental animals remain an important requirement.

To date, laboratory mice remained the animals of choice for experimental facial graft transplantations [9].

This study aimed: 1) to develop and test experimentally the composite facial graft cross-transplantation technique on minipigs; 2) to develop and test on the subjects postoperative therapy and rehabilitation courses, assess the acute rejection diagnostics approach, develop a competent immunosuppressive therapy plan; 3) to test the anesthetic aid used to reduce the risks of perioperative complications.

METHODS

The participants of the experiment carried out three series of facial graft transplantation surgeries on specially selected animals, minipigs, as biological models.

For the experiment, 26 closely related animals were selected: brothers aged from 8 to 24 months, weighing 10–20 kg [10, 11].

The surgeries involved two animals in parallel and took place in a prepared operating room. The participants used standard surgical instruments. An operating microscope was used microscopy stage. As part of the preparation for surgery, we marked the composite facial graft on one animal and, to ensure the maximum possible level of precision, used a template to repeat the same on the other animal. Collecting the grafts, we mobilized the soft tissue components of the flaps while preserving vital structures, keeping the vascular bundles intact to the level of their branching from the external carotid arteries and connecting to the jugular veins, and isolating the facial nerve for subsequent neuroraphy. The bone parts of the grafts were mobilized atraumatically with a piezosurgical tool; after transplantation, they were fastened with Conmet miniplates and miniscrews. Post-surgery, we took biopsy samples dynamically on the 7th, 14th, and 21st days. The samples were used to verify the reparative processes. In case of any signs of rejection, the biopsy samples were collected outside the adopted schedule. We took photos and recorded videos at all stages of the experiment (Fig. 1, 2).

We considered various combinations of flaps with the aim to include the most common flaps designs in our work (Table 1).

Execution of the 1st stage

At the first stage, we carried out experimental facial graft cross-transplantations on five pairs of minipigs (brothers, age — 24 months, weight — 16–20 kg). In the context of these surgeries, we tested and applied the main techniques and flap designs, with the technique application involving all the key stages (Fig. 11–13):

– facial musculocutaneous flap from the buccal, parotid regions;

– composite skin-musculoskeletal flap from the buccal, parotid regions and the lower jaw;

– composite skin-musculoskeletal flap form the paraorbital, buccal, parotid regions and the upper jaw.

Table 1. Flap designs used at different stages of the experiment

Number of animals	Age (months)	Graft design
1st stage		
4	24	Facial musculocutaneous flap from the buccal, parotid regions (Fig. 3, 4)
4	24	Composite skin-musculoskeletal flap from the buccal, parotid regions and the lower jaw (Fig. 5, 6)
2	24	Composite skin-musculoskeletal flap form the paraorbital, buccal, parotid regions and the upper jaw
2nd stage		
2	24	Facial musculocutaneous flap from the buccal, parotid, lower paraorbital regions (Fig. 7, 8)
2	8	Facial musculocutaneous flap from the buccal, parotid, lower paraorbital regions (Fig. 9, 10)
2	24	Facial musculocutaneous flap from the parotid region with auricle and buccal part
2	8	Facial musculocutaneous flap from the parotid region with auricle and buccal part
3rd stage		
4	8	Facial musculocutaneous flap from the buccal and parotid regions, with neuroanastomoses made in the region of facial nerve branches
4	8	Facial musculocutaneous flap from the parotid region with external part of the auricle, buccal region, with neuroanastomoses made in the region of facial nerve branches

Fig. 3. Facial musculocutaneous flap from the buccal, parotid regions (first subject)

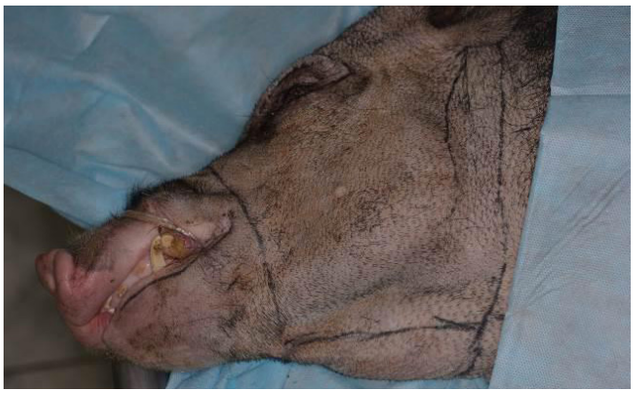

Fig. 4. Facial musculocutaneous flap from the buccal, parotid regions (second subject)

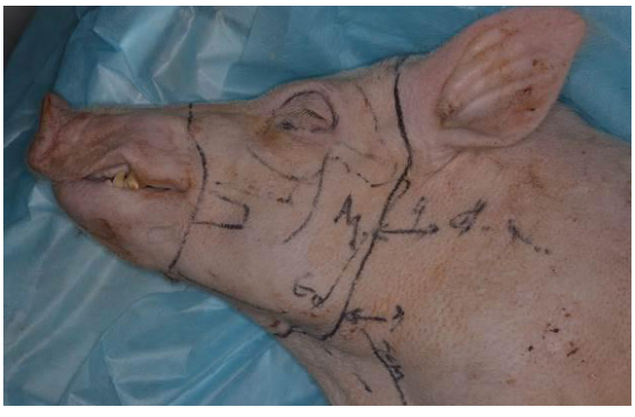

Fig. 5. Composite skin-musculoskeletal flap from the buccal, parotid regions and the lower jaw (first subject)

Fig. 6. Composite skin-musculoskeletal flap from the buccal, parotid regions and the lower jaw (second subject)

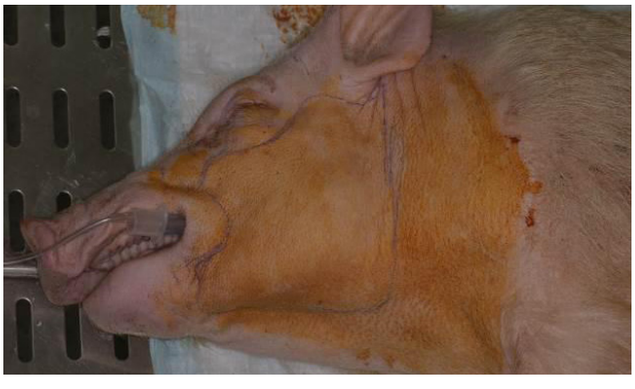

Fig. 7. Facial musculocutaneous flap from the buccal, parotid, lower paraorbital regions (first subject)

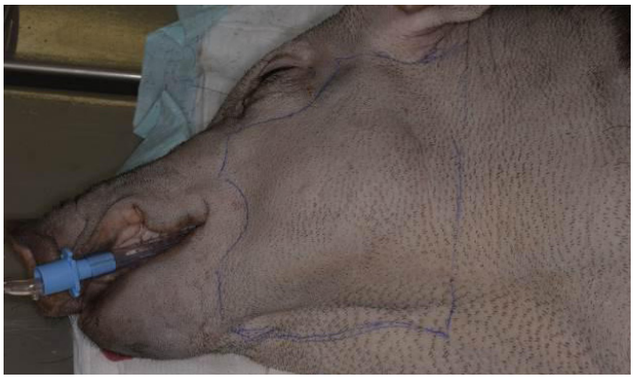

Fig. 8. Facial musculocutaneous flap from the buccal, parotid, lower paraorbital regions (second subject)

Fig. 9. Facial musculocutaneous flap from the parotid region with auricle and buccal part (first subject)

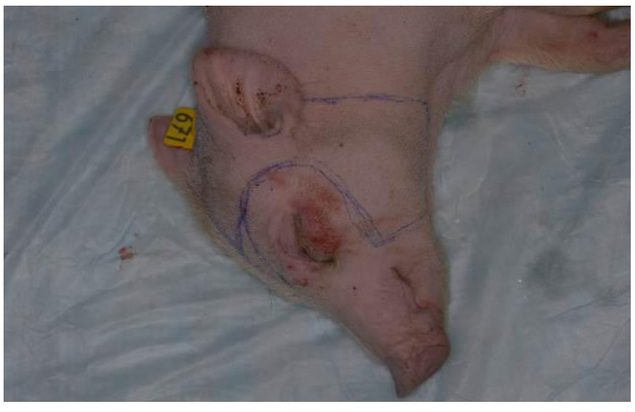

Fig. 10. Facial musculocutaneous flap from the parotid region with auricle and buccal part (second subject)

Surgical interventions were performed under intravenous anesthesia (rometar 0.15 mg/kg + zoletil-100 2 mg/kg) without anesthetic support. The average time of surgery was 14 hours.

Post-surgery, the animals received an antibacterial drug (Baytril for 14 days) and 120 mg of prednisolone i.m. OD throughout the entire follow-up period.

On the 5th day after the operation, two animals developed edema. They were subjected to pulse therapy, and their scheduled prednisolone intake was increased to 240 mg. Five days after, we registered thrombosis of the anastomoses caused by the intensified vascular reaction to hyperergic response of the recipient's body.

Execution of the 2nd stage

At the second stage, we cross-transplanted facial grafts on four pairs of animals (two pairs — brothers, age — 24 months, weight — 20 kg; two pairs — brothers, age — 8 months, weight — 8 kg).

In this experiment, we tested cross-transplantation of the following flap designs:

– facial musculocutaneous flap from the buccal, parotid, lower paraorbital regions;

– facial musculocutaneous flap from the parotid region with auricle and buccal part.

Surgical interventions were performed with anesthetic aid, under intravenous sedation (rometar 0.15 mg/kg, zoletil-100 2 mg/kg, propofol 4 mg/kg, xyla 0.2 ml/kg) and supervision of anesthesiologists. The average time of surgery was 10 hours.

Post-surgery, the animals received 3 ml of Baytril i.m. OD (antibacterial therapy) and 16 mg of dexamethasone i.m. OD (immunotherapy) throughout the entire follow-up period.

Same as at the 1st stage of the experiment, we registered a delayed development of rejection. Clinical manifestations were relieved by pulse therapy (360 mg of solumedrol i.m.).

On the 21st day post-surgery, we collected histological material from the place of fusion of the transplanted flap and the recipient's tissues for histological control.

Execution of the 3rd stage

At the 3rd stage, we cross-transplanted facial grafts on four pairs of animals (four pairs — brothers, age — 8 months, weight — 10 kg). Analysis of the results of the previous stages allowed us to adjust perioperative therapy and the anesthesia protocol. Intra- and post-surgery, we subjected the animals to immunosuppressive therapy [12].

To prevent immediate loss of grafts for immunological reasons, we determined blood group compatibility and performed the microlimphocytotoxic test on the eve of the operation. The fact that each animal was both a donor and a recipient simultaneously was factored in. Individual blood compatibility was checked with the help of room temperature crossmatching.

Based on the results of a series of immunological tests, we made four pairs of animals that underwent a total of eight transplantation surgeries. In each case, the individual compatibility and the microlymphiphocytotoxic tests returned negative.

In this experiment, we continued testing composite flap designs, namely:

– facial musculocutaneous flap from the buccal and parotid regions, with neuroanastomoses made in the region of facial nerve branches;

– facial musculocutaneous flap from the parotid region with external part of the auricle, buccal region, with neuroanastomoses made in the region of facial nerve branches (Fig. 14).

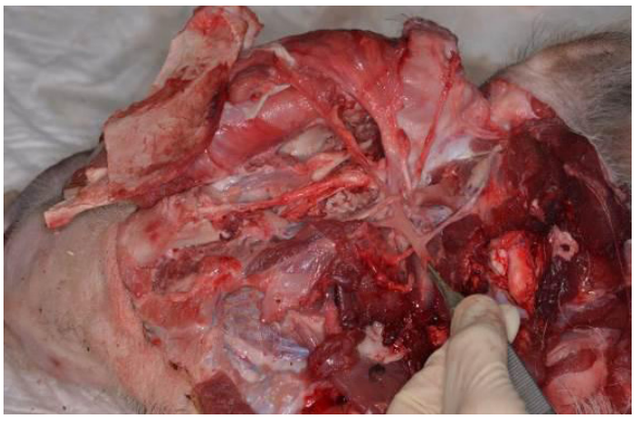

Fig. 11. Intraoperative picture taken after dissection of the composite skin-musculoskeletal flap from the buccal region

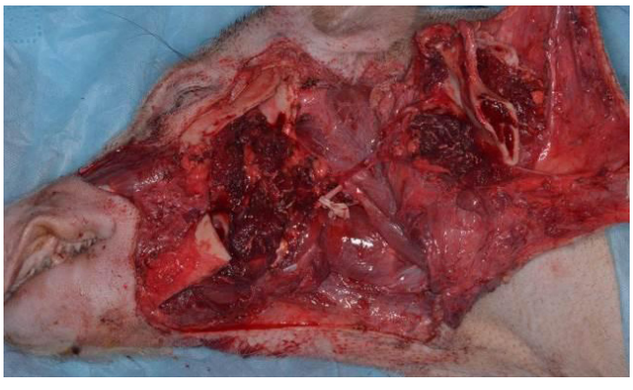

Fig. 12. Intraoperative picture taken after dissection of the composite skin-musculoskeletal flap from the buccal region and the lower jaw

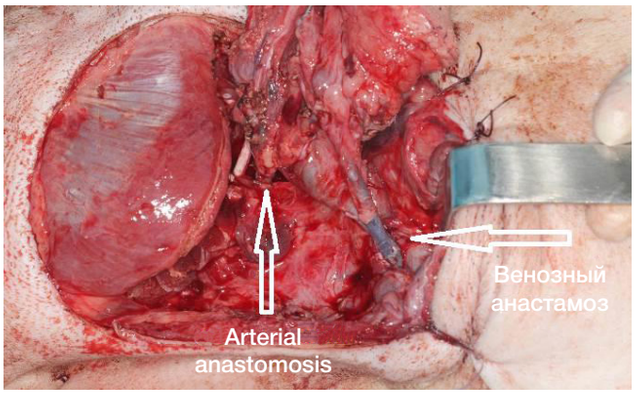

Fig. 13. Intraoperative picture taken after vascular anastomoses were made

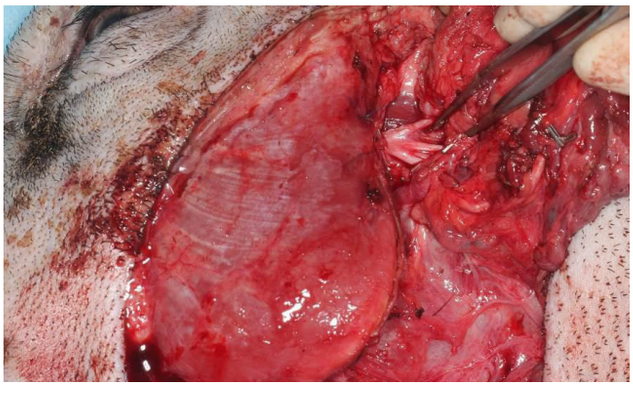

Fig. 14. Intraoperative picture taken after exposition of the facial nerve's trunk and branches

Table 2. Follow-up time at each stage of the experiment

Number of animals	Age (months)	Flap observation time
1st stage		
Two pairs (recipient — donor)	24	36 days
2nd stage		
Two subjects from different pairs	8	30 days (histological confirmation on the 21st day of the primary adhesion process)
3rd stage		
Two subjects from different pairs (administration of tacrolimus)	8	30 days (histological confirmation on the 14th day of the primary adhesion process)

Table 3. Survival of the animals after surgery

Number of animals	Duration
1st stage	
8 out of 10 (80%)	Over 30 days
2nd stage	
7 out of 8 (87.5%)	Over 30 days
3rd stage	
7 out of 8 (87.5%)	Over 30 days

Surgical intervention was performed with anesthetic aid under intravenous sedation (zoletil-100 2 mg/kg, propofol — 4 mg/kg, xyla — 0.2 ml/kg). The average time of surgery was 8 hours.

Based on the additional advice received through consultations with transplantologists and anesthesiologists, we adjusted the drug therapy as follows.

Pre-surgery:

8 hours before intervention — low molecular weight heparins (clexane), s.c.;

antibiotic therapy — 1 ml of interspectin i.v. 30 minutes before the incision.

Intraoperatively, two pairs of subjects received:

0.15 mg/kg of Prograf i.v.;

heparin before the blood flow was resumed.

Post-surgery, experimental models received: antibiotics (1 ml of interspectin per 10 kg of weight i.m. OD) for 14 days with the aim to prevent secondary bacterial complications;

immunosuppressive drug (Solumedrol 160 mg/m) throughout the follow-up period.

We did not register pronounced manifestations of flap rejection post-surgery. The persisting edema were attributed to the volume of intervention and hypersecretion of the salivary gland.

RESULTS

We had the subjects surviving long-term at all stages of the experiment, which indicates humane use of animals. Post-surgery, their vital functions remained unchanged (Table 2). We succeeded in improving the survival rate of models after surgical interventions (Table 3).

Histological examination (Fig. 15) of the recipient–donor boundaries revealed the ongoing primary adhesion process, which prevents acute rejection as it is described in the Banff classification [13, 14].

Figure 15 shows the skin and the subcutaneous tissue, consisting of two fragments, separated by the wound.

The first fragment (recipient) is a skin flap with platysma. The skin is a set of ordinary layers with signs of keratinization and accompanying elements (hair follicles, sebaceous glands). Fatty tissue includes vessels of various sizes. Platysma is of the usual structure, it consists of longitudinal and transverse muscle fibers. In the deep layer, there are glandular structures.

The second fragment is the skin flap with platysma. The skin is a set of ordinary layers with signs of keratinization and accompanying elements (hair follicles, sebaceous glands). Fatty tissue includes vessels of various sizes. The typical platysma of longitudinal and transverse muscle fibers has narrow strands of granulation tissue penetrating it. The vessels contain form elements.

The wound is a narrow slit filled with granulation tissue of low cellularity. The granulation tissue mainly consists of small capillaries and interlayers of connective tissue with thin fibrils. It is practically not infiltrated with polymorphonuclear leukocytes (neutrophils), lymphocytes. They are found only in the surface layer under a patch of necrotic epidermis. Along the wound slit, infiltration with multinucleated cells can only be seen from the side of the first fragment.

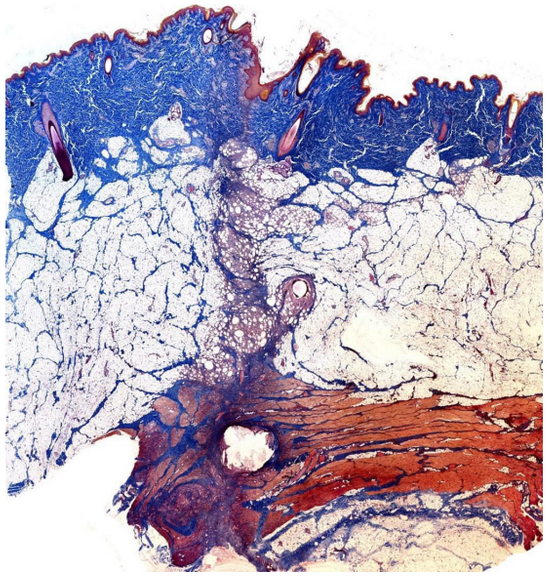

Fig. 15. Place of fusion of the flap with the recipient's tissues on the scanned image of the histological specimen

Table 4. Flap survival depending on the type of antibacterial and immunosuppressive therapy selected

Immunotherapy	Result
1st stage	
Antibacterial (ceftriaxone i.m. OD) + immunosuppressive therapy (prednisolone 120 mg or 240 mg as pulse therapy in case of rejection)	Two flaps (out of 10) from different pairs: survival without signs of acute rejection up to 36 days, development of delayed acute rejection followed by a pulse therapy relief attempt
2nd stage	
Antibacterial therapy (enrofloxacin i.m. OD) and immunotherapy (16 mg of dexamethasone i.m. OD, 32 mg of dexamethasone OD as pulse therapy in case of rejection)	Two flaps (out of 8) from different pairs — engraftment on the 21st day, with arrested acute rejection in the postoperative period
3rd stage	
Antibacterial therapy (lincomycin + spectinomycin i.m. OD) and immunotherapy (tacrolimus — intraoperative i.v., methylprednisolone i.m.)	Two flaps (out of 8) from different pairs — engraftment on the 14th day without signs of rejection

Table 4 shows the results of graft retention depending on the therapy regimens in the peri- and postoperative periods. It should be noted that the response is more effective in the cases where acute rejection reactions were purposefully relieved.

DISCUSSION

Even with the histological analysis confirming graft healing, it is necessary to closely observe the dynamics of the processes post-surgery and adjust the immunosuppressive therapy regimen with minimum possible delay following registration of signs of the acute tissue rejection reaction.

Having analyzed the results of our experiment and considered the cases of development of acute graft rejection, we concluded that it is necessary to continue development and testing of the immunosuppression regimen, which is consistent with the results other researchers have arrived at [15]. Another group of researchers has discovered that the features of the composite graft play a role in the development of rejection in one of its components [16], which leads to loss of the skin part of the flap while its muscle components remains.

Thus, the question is raised about the need to select objective methods for diagnosing the state of all components of the flap. Also, compared to single organ transplantation, surgeries involving composite grafts require greater attention to the specific features of such grafts.

CONCLUSIONS

The experimentally tested composite facial graft cross-transplantation technique allows all members of the team (surgeons, anesthesiologists, transplantologists, immunologists) to practice and improve their skills involved in the preparation, conduct of the surgery and postoperative rehabilitation of face transplant patients. Extended anesthetic aid was registered to decrease the operating time and improve survival rate of the subjects post-surgery.

The immunosuppressive therapy applied at this stage of the experiment requires further adjustment and testing to reduce the risk of development of acute or chronic rejection.

The emphasis on the unique features of composite grafts may allow additional, more specific treatment, which can multiply the life expectancy of patients with such grafts. Given the above, it is worth considering the possibility of using alemtuzumab perioperatively in addition to the plan typically followed in the context of transplantation surgeries.

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EFFECTIVENESS OF IMMOBILIZED PROBIOTICS FOR COMPLEX THERAPY OF NOVEL CORONAVIRUS INFECTION COVID-19 IN HOSPITAL SETTINGS

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Taking into account the gut–lung microbiota axis, the new probiotic treatment methods for COVID-19 are currently being discussed. There are effective medicinal preparations of domestic manufacture in the Russian Federation, the immobilized probiotics. The study was aimed to determine the effectiveness of the mixed immobilized probiotic containing the immobilized *B. bifidum* and lactobacilli *L. plantarum* (100 million CFU per dose) or the simple immobilized probiotic containing the immobilized *B. bifidum* (500 million CFU per dose) in the complex therapy of patients with COVID-19. During the open-label, prospective, observational study 70 patients with confirmed diagnosis of COVID-19 received complex treatment which included the immobilized probiotics. All patients were discharged from the hospital with imporoved health status, as well as with improved instrumental and laboratory indicators: body temperature returned to normal in all patients; shortness of breath, cough, feeling of chest tightening, myalgia and headache disappeared; the patients regained sense of smell and taste; the weakness decreased or disappeared (pathognomic symptom for COVID-19). The dynamics of clinical, laboratory and instrumental indicators reflecting the course of the novel coronavirus infection demonstrates the effectiveness of the used complex therapy. The immobilized probiotics may be recommended for the complex treatment of patients with COVID-19.

Keywords: coronavirus infection, COVID-19, immobilized probiotics, bifidobacterium, lactobacillus

Author contribution: Bomshteyn NG, Bolotov YuV — study concept and design, data acquisition and processing, manuscript writing; Kim IA, Trukhin DV — study management, manuscript editing.

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ЭФФЕКТИВНОСТЬ СОРБИРОВАННЫХ ПРОБИОТИКОВ В КОМПЛЕКСНОМ ЛЕЧЕНИИ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19 В УСЛОВИЯХ СТАЦИОНАРА

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С учетом оси «кишечник–легкие–микробиота» в настоящее время обсуждают потенциально новые методы лечения инфекции COVID-19 с применением пробиотиков. В Российской Федерации существуют эффективные отечественные препараты — сорбированные пробиотики. Целью исследования было определить эффективность включения в комплексную терапию больных COVID-19 поликомпонентного сорбированного пробиотика, содержащего сорбированные *B. bifidum* и лактобактерии *L. plantarum* (100 млн КОЕ в пакете), или монокомпонентного сорбированного пробиотика, содержащего сорбированные *B. bifidum* (500 млн КОЕ в капсуле). В открытом проспективном наблюдательном исследовании 70 пациентам с подтвержденным диагнозом COVID-19 проводили комплексное лечение с включением сорбированных пробиотиков. Все пациенты выписаны из стационара с улучшением состояния, а также инструментальных и лабораторных показателей: у всех пациентов нормализовалась температура, исчезли одышка, кашель, ощущение заложенности в грудной клетке, миалгия, головная боль, восстановились обоняние и вкусовые ощущения, уменьшилась или исчезла слабость (характерный симптом COVID-19). Динамика клинических, лабораторных и инструментальных показателей, отражающих течение новой коронавирусной инфекции, указывает на эффективность проводимой комплексной терапии. Сорбированные пробиотики могут быть рекомендованы к применению в комплексном лечении пациентов с COVID-19.

Ключевые слова: коронавирусная инфекция, COVID-19, сорбированные пробиотики, бифидобактерии, лактобактерии

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The World Health Organization (WHO) on February 11, 2020 gave the disease caused by the novel coronavirus the name COVID-19 (Coronavirus Disease 2019). The emergence of COVID-19 set the healthcare specialists the task of rapid diagnosis and medical care provision. Under existing conditions of the fast spread of infection and limited evidence of the COVID-19 treatment, the WHO recommendations allow one to prescribe drugs off-label in accordance with the ethical standards [1].

Taking into account the gut–lung microbiota axis, which is responsible for maintaining homeostasis, the new probiotic treatment methods for COVID-19 are currently being discussed [2, 3]. Gut microbiota contributes to the course of COVID-19 due to its relationship with immune system and lungs. Interaction between gut and lungs may affect the severity of COVID-19 [4].

There are effective medicinal preparations of domestic manufacture in the Russian Federation, probiotics, which are considered a distinct group of immobilized probiotics by the State Pharmacopoeia of the Russian Federation. Unlike other probiotics, the immobilized probiotics contain microcolonies

of bifidobacteria on the activated carbon particles, which enable their targeted delivery to parietal biotopes of intestines, therefore increasing the effectiveness of the disease treatment and prevention [5].

The immobilized probiotics are classified into simple and mixed probiotics. There are interchangeable dosage forms of the medications (oral powder and capsules).

The simple probiotic (SIP) contains at least 500 million colony forming units (CFU) of bifidobacteria *Bifidobactrium bifidum* immobilized on the activated carbon particles per capsule, along with excipient (lactose monohydrate up to 0.20 g). It exhibits anti-infective, antitoxic, antioxidant and antidiarrheal effect. According to the product instruction, SIPs are used as part of complex therapy of acute respiratory viral infections and flu, in patients with secondary immune deficiencies, severe infectious inflammatory and purulent septic diseases; for treatment of diarrhea of different etiology; in patients with dysbioses of different etiology, including those resulting from the use of antibiotics.

The assessment of the master seed sensitivity showed that the contained in the SIP *B. bifidum 1* strain is sensitive to azithromycin [6], which should be considered when prescribing SIP together with azithromycin. In such a situation the daily dose of the immobilized probiotic should be increased.

The sachet of mixed probiotic (MIP) contains *Bifidobacterium bifidum 1* immobilized on the activated carbon particles, and *Lactobacillus plantarum* 8P-A3 (at least 500 million CFU of each strain), together with lactose monohydrate (up to 0.85 g).

According to the product instruction, MIPs are used in patients with both viral and bacterial respiratory infections, for restoration of respiratory and gut ecosystems during the period of convalescence, and in patients with dysbioses of different etiology, including those resulting from the use of antibiotics.

The capacity of oral immobilized probiotics to improve the nasopharyngeal microbiota functioning has been reported [7].

The active components of immobilized probiotics are considered benign, since the *B. bifidum* bifidobacteria and *L. plantarum lactobacilli* are the main representatives of the normal resident human microbiota [5].

The study was aimed to determine the effectiveness of the mixed immobilized probiotic containing the immobilized *B. bifidum* and lactobacilli *L. plantarum* (100 million CFU per dose) or the simple immobilized probiotic containing the immobilized *B. bifidum* (500 million CFU per dose) in the complex therapy of patients with novel coronavirus infection.

METHODS

The open-label, prospective, observational study included 70 patients admitted to the Scientific and Clinical Center of Otorhinolaryngology of the Federal Medico-Biological Agency of the Russian Federation from April 25 to May 26, 2020. Inclusion criteria: confirmed diagnosis of the novel coronavirus infection COVID-19 (positive SARS-CoV-2 RNA testing results); moderate course of the disease.

All patients underwent physical examination, laboratory and instrumental testing. The course of the disease was assessed during the physical examination on daily rounds and via control of laboratory and instrumental testing results within the recommended time-frame: complete blood count (CBC), blood chemistry tests, C-reactive protein test (CRP), chest computed tomography (chest CT), pulse oximetry, thermometry. The assessment of the lung damage severity on CT was carried out in accordance with the temporary guidelines [1].

Throughout the study the patients received standard COVID-19 therapy in accordance with the temporary guidelines of the Ministry of Health of the Russian Federation (version 6) [1], which included hydroxychloroquine, azithromycin, symptomatic and supportive care (if medically necessary).

In addition 30 patients (group 1) received SIP, 2 capsules 4 times daily within 14 days after admission to hospital, and 40 patients (group 2) received MIP, 2 sachets 3 times daily since the 2nd week of hospital stay for 10–14 days.

The effectiveness of the therapy was assessed based on the dynamics of clinical symptoms, laboratory and instrumental testing results, and the length of hospital stay.

Statistical processing of the results was carried out using the STATISTICA 9.0 software (StatSoft Inc.; USA).

Quantitative variables were presented as median (Me), lower and upper quartiles. The discrete characters were presented as event rate (number of cases proportional to the number of observations, %).

RESULTS

The main characteristics of the patients are presented in Table 1. Since the effectiveness of probiotics is not related to gender, and due to the features of hospitalization during the pandemic, most patients who have received treatment using the discussed scheme are males (70%).

The following symptoms were observed in patients of both groups upon admission: elevated body temperature (within the range of 37.3–38.0 °C in 60 and 80% of patients of group 1 and group 2 respectively, and above 38 °C in 40 and 20% of patients respectively), dry cough or cough with little phlegm, the feeling of chest tightening, shortness of breath, weakness, myalgia, headache. Rhinitis and the loss of smell and taste were observed in 40–60% of patients in group 1, and in 15–20% of patients in group 2. No nausea, vomiting or diarrhea were detected in any of the patients.

On admission based on the empirical indicators of the visual scale (the average amount of lung tissue thickening, bilateral) most patients of group 1 (60%) were diagnosed with moderate lung lesion on CT (CT2), and 40% of the patients were diagnosed with mild lung lesion (CT1). Among patients of group 2, moderate lung lesion (CT2) was diagnosed in 37.5%, and mild lung lesion (CT1) was diagnosed in 62.5%. It should be noted that the differences in the lung damage rate in patients of studied groups were not significant ($\chi^2 = 2.64$, $p = 0.104$, the critical value $\chi^2 = 3.84$ at $p = 0.05$).

The oxygen saturation values obtained by pulse oximetry (SpO_2) in patients of group 1 were 97–96% (all patients), in patients of group 2 they were 97% or less (60% of patients), and 98% (40% of patients).

The CRP level was within the reference range in 20% of patients of group 1, and 40% of patients of group 2, exceeded the reference value by 1.8–14.8 times in 80% of patients of group 1, and by 2.3–19.7 times in 60% of patients of group 2.

The complete blood count (CBC) revealed the decrease in the number of leukocytes ($3.12–3.60 \times 10^9$/L) in 60% of patients of group 1. Alterations in white blood cell count were detected in 10% of patients. The other parameters' values were within the reference ranges in all patients. Complete blood count in patients of group 2 revealed alterations in the percentage of certain white blood cell types and the total white blood cell count (slight decrease) in 15% of patients. In the rest of the patients (75%), all indicators were within normal range.

The blood chemistry test values (urea, creatinine, bilirubin, glucose, albumin, electrolytes) in all patients of group 1 were within the reference range. In 10% of patients the elevated values of ALT and AST were observed, and in other 90%

Table 1. Characteristics of patients included in the study

Parameter	Group 1 (*n* = 30)	Group 2 (*n* = 40)
Average age, years (Me [lower quartile; upper quartile])	51 [45; 64]	47 [32; 53]
Principal diagnosis		
Novel coronavirus infection COVID-19 (confirmed), moderate course, U07.1, abs. (%)	30 (100)	40 (100)
Complications		
Community-acquired pneumonia, bilateral polysegmental, J18.9, abs. (%)	30 (100)	40 (100)
Comorbidities, abs. (%)		
Hypertensive heart disease	12 (40)	11 (27.5)
Type 2 diabetes mellitus	2 (6.7)	4 (10)
History of the gastrointestinal tract disorders	9 (30)	8 (20)
History of chronic bronchitis	3 (10)	5 (12.5)
Bronchial asthma	1 (3,3)	–
Chronic sinusitis	–	2 (5)
Clinical parameters upon admission, abs. (%)		
Elevated body temperature	30 (100)	40 (100)
Feeling of chest tightening	30 (100)	40 (100)
Shortness of breath	30 (100)	40 (100)
Fatigue, weakness	30 (100)	40 (100)
Myalgia	30 (100)	40 (100)
Headache	30 (100)	40 (100)
Rhinitis	9 (30)	6 (15)
Loss of smell and taste	10 (33.3)	8 (20)
Pulmonary lesions severity on CT, abs. (%)		
CT 1	12 (40)	25 (62.5)
CT 2	18 (60)	15 (37.5)

of patients these values were within the reference range. The blood chemistry test values in patients of group 2 (urea, creatinine, ALT, AST, bilirubin, glucose, albumin, electrolytes) were within the reference range in 85% of patients, and 15% of patients had elevated transaminase level. In patients with diabetes mellitus of both groups, the glucose level was within the range typical for compensated state.

The clinical parameters dynamics along with the length of hospital stay for group 1, which received SIP since the day of admission to hospital, is presented in Table 2.

In most patients, the body temperature decrease was observed on day 10 of hospital stay, body temperature dropped to normal on days 14–15, and the cough and the feeling of chest tightening decreased and disappeared within the same period. In most patients, the shortness of breath disappeared within 6 days of treatment, and on day 9 no shortness of breath was observed in all patients. Myalgia and headache decreased and disappeared within the same period. In patients who experienced the loss of smell and taste, the sense of smell and taste recovered on days 10–14, and rhinitis disappeared during the same period. On the day of discharge the weakness disappeared in 70% of patients, and decreased in 30% of patients.

Thus, under complex treatment, on the day of discharge all patients had no elevated body temperature, shortness of breath, cough, feeling of chest tightening, myalgia, headache or rhinitis. The sense of smell and taste recovered, and the weakness disappeared in most patients.

All patients had regular and well-formed stool during the whole observation period.

On the day of discharge the mild lung lesion on CT (CT1) indicating the clinical improvement was diagnosed in all patients. The oxygen saturation and CRP level values were back to normal.

The dynamics of complete blood count showed the increase in the number of leukocytes in all patients, who had the decreased number of leukocytes upon admission. On the day of discharge only one patient had low number of leukocytes compared to reference value, however, that value was close to the lower threshold of reference range.

On the day of discharge from hospital the blood chemistry test values were within the reference range in all patients.

Table 2. Clinical parameters dynamics and the length of hospital stay for the group of patients who received SIP

Parameter	Day of improvement*	Day of the symptom disappearance *
Elevated body temperature	10 [9; 11]	15 [14; 16]
Shortness of breath	4 [4; 5]	6 [5; 7]
Feeling of chest tightening	10 [9; 11]	15 [14; 16]
Cough	10 [9; 11]	15 [14; 16]
Myalgia	4 [3; 5]	6 [5; 7]
Headache	4 [3; 5]	6 [5; 7]
Length of hospital stay (days)*	18 [17; 19]	

Note: * — median [lower quartile; upper quartile].

Table 3. Clinical parameters dynamics and the length of hospital stay for the group of patients who received MIP

Parameter	Number of patients exhibiting the symptom when starting MIP, abs. (%)	Day of the symptom disappearance after starting MIP
Elevated body temperature	34 (85)	4 [3; 5]
Shortness of breath	34 (85)	4 [3; 6]
Feeling of chest tightening	37 (92.5)	5 [4; 6]
Cough	37 (92.5)	5 [4; 6]
Myalgia	20 (50)	4 [3; 5]
Headache	20 (50)	4 [3; 5]
Length of hospital stay (days)*	18 [17; 21]	

Note: * — median [lower quartile; upper quartile].

The clinical parameters dynamics along with the length of hospital stay for group 2, which received MIP since the 2nd week of hospital stay, is presented in Table 3.

The body temperature dropped to normal on days 3–5 of exposure to MIP in most patients, myalgia and headache disappeared within the same period. Shortness of breath disappeared, feeling of chest tightening and cough within 6 days of treatment. In patients who experienced the loss of smell and taste, the sense of smell and taste recovered on days 4–5 of treatment with MIP, rhinitis disappeared during the same period. Weakness disappeared on days 5–14 of exposure to MIP, more often on days 7–8. On the day of discharge no weakness was observed in all patients.

Thus, under complex treatment, on the day of discharge all patients had no elevated body temperature, shortness of breath, cough, feeling of chest tightening, myalgia, headache, weakness and rhinitis. The sense of smell and taste recovered.

All patients had regular and well-formed stool during the whole observation period.

The dynamic changes of chest CT based on the empirical visual assessment data are presented in Table 4.

Prior to starting MIP, the changes of CT findings were observed in all patients: no clinical worsening compared to the CT scan results obtained on admission was detected in 25% of patients, and 75% of patients showed signs of clinical improvement. Under complex treatment with the use of MIP, on the day of discharge the mild lung lesion on CT was diagnosed in all patients, indicating the clinical improvement. The oxygen saturation and CRP level values were back to normal.

When starting MIP, only one patient showed slight decrease in the number of leukocytes, the other patients' values were within the reference range. On the day of discharge the complete blood count values were within the reference range in all patients. The blood chemistry test values also corresponded to reference values, except for patients with diabetes mellitus, whose glucose level was elevated, but was within the range typical for compensated state.

The use of immobilized probiotics revealed no side effects, adverse events or adverse reactions.

DISCUSSION

In the context of sharp rise in the incidence of the novel coronavirus infection COVID-19 resulting in severe patients' condition and sometimes being lethal, and the lack of precise treatment schemes, there was an urgent need for medical care improvement. Therefore, the use of the medicinal preparations of domestic manufacture, the immobilized probiotics with high safety profile and proven effectiveness regarding the acute respiratory illnesses, for complex treatment seemed natural enough. Moreover, the capability of immobilized probiotics to prevent and neutralize the adverse effects of antibacterial therapy is well known [5, 7]. To avoid the excess load and the divergent effect on the gut microbiota and human body, the possibility to use the simple probiotic containing the microcolonies of *Bifidobacterim bifidum* in terms of effectiveness dramatically different from preparations containing single cells of bifidobacteria of this species during the acute period of the disease was considered important [5]. The treatment using the specially selected combination of Bifidobacterim bifidum microcolonies with the *Lactobacillus plantarum* species (MIP) was started since the 2nd week of hospital stay [5, 7]. During the observational study the complex treatment results were analyzed in each of two groups of patients who received SIP and MIP in accordance with different treatment schemes.

Despite the small sample size and the lack of comparison group, when analyzing the results of COVID-19 patients' complex treatment, which included SIP and MIP, the general state improvement, as well as the improvement of the laboratory and instrumental testing results stood out in all patients. Under complex treatment on the day of discharge all patients had no elevated body temperature, shortness of breath, cough, feeling of chest tightening, myalgia, and headache. The weakness, being a pathognomic symptom for COVID-19, disappeared in all patients who received MIP, and in most patients, who received SIP. All patients of the studied groups, who exhibited the smell and taste loss upon admission, regained sense of smell and taste during the 2nd week of treatment. Even though the recovery of smell and taste is typical for the 2nd–4th week from the beginning of the disease, it never occurs in all patients. Therefore the fact of smell and taste recovery under treatment with immobilized probiotics merits consideration and may be subject to further research.

The patients received essential therapy which adversely affected the gut microbiota, however, none of them complained of flatulence, abdominal pain, and diarrhea. All patients had regular, well-formed stool during the whole observation period, which could be due to positive effect of MIP and SIP on the gut microbiota and better tolerability of essential therapy.

Table 4. Dynamic changes of chest CT based on the empirical visual assessment data

Upon admission		During treatment (days 2–3 of taking MIP)		On the day of discharge	
CT results	Number of patients, abs. (%)	CT results	Number of patients, abs. (%)	CT results	Number of patients, abs. (%)
CT 2	15 (37.5)	CT 2	12 (30)	CT 2	0
CT 1	25 (62.5)	CT 1	28 (70)	CT 1	40 (100)

CONCLUSION

Upon the novel coronavirus infection COVID-19 complex treatment with the use of medication containing immobilized *B. bifidum 1* and *L. plantarum* 8P-A3 or immobilized B. *bifidum 1,* all patients demonstrated the improvement of clinical, laboratory and instrumental indicators reflecting the course of the disease, which indicated the effectiveness of the therapy. The immobilized probiotics may be recommended for the complex treatment of patients with COVID-19.

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ADAPTATION TO INTERMITTENT HYPOXIA: DYNAMICS OF BLOOD OXYGEN SATURATION AND SOME HEMATOLOGICAL PARAMETERS

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Adaptation to hypoxia is an important object of medical research. The aim of this study was to investigate the dynamics of blood oxygen saturation (SpO_2), arterial blood pressure (BP), red blood cells, reticulocytes, hemoglobin and erythropoietin (EPO) concentrations during intermittent hypoxic training (IHT). The study was conducted in 11 healthy male volunteers; 2 regimens were tested: 11 and 14 days of IHT at F_IO_2 = 9%. Exposure to the hypoxic gas mixture caused a reduction in SpO_2 by an average of 20.4% ($p < 0.05$), a 22% increase in the heart rate ($p < 0.05$) and a 4.5% decrease in diastolic BP ($p < 0.05$) relative to the initial levels. After 11 days of IHT training, the reticulocyte count was increased by 16.6% ($p < 0.05$), and there was a distinct tendency to elevated red blood cells ($p > 0.05$) and hemoglobin ($p > 0.05$). EPO concentrations declined by 44.2% ($p < 0.05$) relative to the initial level. Extending the regimen to 14 days resulted in a 3.9% increase in red blood cell count ($p < 0.05$) and a 4.7% elevation of hemoglobin concentrations ($p < 0.05$), accompanied by the recovery of the initial reticulocyte count. The applied 2-week IHT regimen resulted in the increased red blood cell count and elevated hemoglobin, suggesting an improvement in the oxygen-carrying capacity of the blood. The proposed regimen can be used to improve physical performance of individuals working in extreme environmental conditions.

Keywords: intermittent hypoxic training, blood oxygen saturation, erythropoietin, hemoglobin, red blood cells, reticulocytes, arterial blood pressure.

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Compliance with ethical standards: the study was approved by the Ethics Committee of Federal Research Clinical Center of FMBA (Protocol № 1 dated February 7, 2019) and conformed with the principles of biomedical ethics laid out in the Declaration of Helsinki (the 1964 version and subsequent updates); voluntary informed consent was obtained from each study participant.

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АДАПТАЦИЯ К ИНТЕРВАЛЬНОЙ ГИПОКСИИ: ДИНАМИКА НАСЫЩЕНИЯ КРОВИ КИСЛОРОДОМ И НЕКОТОРЫХ ГЕМАТОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ

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Адаптация к гипоксии является одной из актуальных проблем медицины. Целью работы было изучить динамику насыщения крови кислородом (SpO_2), артериального давления (АД), показателей красного ростка крови и уровня эритропоэтина (Эпо) в процессе интервальных гипоксических тренировок (ИГТ). При участии 11 мужчин-добровольцев проведено две серии исследований с 11- и 14-суточным курсом ИГТ при F_IO_2 = 9%. Дыхание воздухом с пониженным PO_2 приводило к уменьшению SpO_2 в среднем на 20,4% ($p < 0,05$), увеличению частоты сердечных сокращений на 22% ($p < 0,05$) и снижению диастолического АД на 4,5% ($p < 0,05$) по отношению к исходным значениям. После 11-суточного курса ИГТ наблюдали увеличение в крови числа ретикулоцитов на 16,6% ($p < 0,05$), тенденцию к увеличению числа эритроцитов ($p > 0,05$) и содержания гемоглобина ($p > 0,05$). Уровень Эпо по сравнению с исходной величиной снижался на 44,2% ($p < 0,05$). Увеличение курса ИГТ до 14 суток привело к повышению числа эритроцитов на 3,9% ($p < 0,05$) и содержания гемоглобина на 4,7% ($p < 0,05$), что сопровождалось уменьшением числа ретикулоцитов до исходного уровня. Двухнедельный курс ИГТ приводит к увеличению в крови числа эритроцитов и содержания гемоглобина, что указывает на повышение кислородной емкости крови. Разработанный протокол ИГТ может быть использован при подготовке специального контингента лиц к работам с повышенной физической нагрузкой в экстремальных условиях окружающей среды.

Ключевые слова: интервальные гипоксические тренировки, насыщение крови кислородом, эритропоэтин, гемоглобин, эритроциты, ретикулоциты, артериальное давление.

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Exploring the impact of the reduced partial pressure of oxygen (PO_2), i.e. hypoxic hypoxia, on the human body is an important area of medical research. Depending on the degree of environmental PO_2 reduction, hypoxia can either provoke pathology or exert a revitalizing effect [1–10]. The studies by Felix Z. Meerson generated a vast array of data suggesting that

adaptive hypoxic training could improve overall endurance and tolerance of hypoxia or other harsh environmental conditions, including extreme cold and physical strain; Meerson's works provided a rationale for his concept of cross adaptation, the general mechanism of adaptation and prophylaxis [11, 12].

Success in decoding the molecular mechanism of oxygen homeostasis has become one of the major advances in biology made in the last 3 decades. The key regulators of oxygen homeostasis are hypoxia-inducible factors (HIFs) [13], of which HIF-1 is highly crucial and well-studied. HIF-1 is a heterodimer composed of an oxygen-dependent subunit HIF-1α and a structural subunit HIF-1β. The concentration and stability of HIF-1α and its transcriptional activity are directly dependent on PO_2 in the cell [14, 15]. Under reduced PO_2, HIF-1α initiates a cascade of gene-mediated cellular and systemic reactions conducive to delivering enough oxygen to tissues and subsequent oxygen uptake. HIF-1 and HIF-2 stimulate production of erythropoietin (EPO) by the kidneys. EPO is a hormone that regulates production of red blood cells in the bone marrow [16]; in turn, red blood cells carry oxygen from the lungs to other tissues.

This theoretical thesis is in good agreement with the experimental data demonstrating that long exposure to an altitude > 2,200 m leads to an increase in serum EPO concentrations [17] and altitude acclimatization is characterized by polycythemia, elevated hemoglobin and increased oxygen-carrying capacity of the blood [1, 3, 18–20]. However, the associations between EPO levels, hematological parameters of red blood cells and physiological effects of hypoxia may not always be very pronounced in intermittent hypoxic training (IHT), which is used to stimulate adaptation to hypoxia. For example, no increase in EPO concentrations, hematological parameters of red blood cells or improved endurance performance were observed in distance runners undergoing a 4-week normobaric IHT program (5 min of normoxia followed by 5 min of hypoxia, 70 min per session, 5 times a week; F_IO_2 = 12% at week 1, F_IO_2 = 11% at week 2, F_IO_2 = 10% at weeks 3 and 4) [21]. Another study conducted in athletes found no significant differences in the hematological parameters of red blood cells and hemoglobin mass at baseline and after 4 weeks of IHT in a hypobaric chamber (3 h a day, 5 days a week, pressure equivalent to that at 4,000–5,500 m), although there was a twofold increase in EPO concentrations after exposure to the hypoxic environment [22]. Another study reported complement activation, increased phagocytic activity of neutrophils and elevated immunoglobulins in 10 healthy male volunteers undergoing a 2-week normobaric IHT program (5 min of hypoxia followed by 5 min of normoxia, 4 times a day [23]. However, the positive effects of IHT observed in the cited study were not accompanied by EPO elevation, increased erythrocyte count or heightened hemoglobin concentrations. One more publication reported the absence of changes in hematocrit and hemoglobin concentrations in 9 healthy males undergoing a 12-day normobaric IHT program (2h a day at F_IO_2 ~13%) [24]; however, by day 5 their reticulocyte count was elevated.

Considering that IHT is widely used in clinical, sports, aviation and space medicine [7, 8, 25–27], it is important to study its effects on the human body, the underlying mechanisms, the efficacy of different IHT regimens and approaches to their optimization [28]. The aim of this study was to investigate changes in oxygen saturation, arterial blood pressure, hematological parameters of red blood cells and EPO concentrations throughout a 2-week IHT program.

METHODS

The study was carried out on 11 apparently healthy male volunteers aged 21–32 years (the mean age was 25.3 ± 1.5 years; the mean weight, 81.5 ± 3.3 kg; the mean height, 180.4 ± 2.2 cm). The following inclusion criteria were applied: approval by the medical board and voluntary consent to participate.

IHT sessions were conducted using a Bio-Nova-204 system for hypoxic therapy (Bio-Nova; Russia) that allows delivering a hypoxic gas mixture to 2 patients at a time. During the sessions, the participants remained seated. The mixture was delivered through a mask pressed tightly against the face, in a well-ventilated room for physiological tests involving humans. The sessions were administered on a daily basis; each session lasted 60 min and consisted of 6 cycles of breathing the hypoxic gas mixture (5 min) followed by breathing ambient air (5 min). Thus, each session included six 5-minute long periods of inhaling the hypoxic has mixture, and the total duration of hypoxic exposure was 30 min. During the first IHT session, F_IO_2 was 10%, which corresponds to P_IO_2 ~76 mmHg. During the second and the remainder sessions, F_IO_2 was 9% (P_IO_2 ~68.5 mmHg). In the first part of the experiment, an 11-day regimen was applied to 5 participants; in the second part, the regimen was extended to 14 days and was administered to 6 participants.

During the sessions, the physiological and subjective responses of the participants to the inspired low-oxygen mixture were closely monitored. Systolic (SBP) and diastolic (DBP) blood pressures, SpO_2 and heart rate (HR) were measured at baseline and during the inhalation of the hypoxic mixture using a PVM-2703 monitor (Nihon Kohden Corporation; Japan).

For blood tests, fasting blood samples were drawn from a basilic vein in the morning prior to commencing the program and upon completion of the first (11 days) and second (14 days) parts of the experiment. Measurements were done using an automated hematology analyzer XN-3000 (Sysmex Corporation; Japan). EPO was measured using an Immulite 2000 XPi analyzer (Siemens; Germany) before starting the 11-day regimen and upon its completion.

Prior to and after completing the extended 14-day IHT regimen, a functional test previously described in [29] was

Table 1. Oxygen saturation (SpO_2), heart rate (HR), systolic (SBP) and diastolic (DBP) pressures in the participants during hypoxic gas breathing

Stage of the experiment	SpO_2, %	HR, min^{-1}	SBP mmHg	DBP, mmHg
Before IHT	97.0 ± 0.5	71.7 ± 4.0	127.6 ± 3.1	80.2 ± 1 .8
IHT № 1	75.3 ± 1.3*	89.0 ± 4.3*	125.3 ± 6.1	77.8 ± 1.3
IHT № 4	76.5 ± 3.2*	90.6 ± 1.3*	124.7 ± 7.3	80.7 ± 5.4
IHT № 8	78.6 ± 2.3*	85.3 ± 4.7*	127.5 ± 7.0	76.7 ± 2.6
IHT № 11	78.1 ± 1.9*	84.6 ± 5.5*	123.4 ± 4.8	73.7 ± 1.8*
IHT № 14	77.6 ± 2.6*	86.8 ± 4.1*	127.8 ± 4.8	74.2 ± 2.8*

Note: IHT — intermittent hypoxic training; * — $p < 0.05$ for comparisons with pretraining data

Table 2. Hematological parameters of red blood cells and erythropoietin levels before and after the IHT program

Parameter	11-day IHT regimen		14-day IHT regimen	
	Before IHT	After IHT	Before IHT	After IHT
Red blood cell count, $\times10^{12}$/L	4.85 ± 0.38	5.0 ± 0.32	5.1 ± 0.17	5.3 ± 0.23*
Hemoglobin, g/L	138.2 ± 5.38	143.8 ± 7.91	150.2 ± 4.2	157.3 ± 5.73*
Erythropoietin, mME/ml	7.35 ± 2.5	4.1 ± 0.96*	–	–
Hematocrit, %	42.4 ± 2.4	43.3 ± 2.6	45.4 ± 1.13	46.4 ± 1.83
Reticulocyte count, $\times10^{9}$/L	71.7 ± 4.2	83.6 ± 6.7*	73.9 ± 5.2	68.9 ± 3.5

Note: * — $p < 0.05$ for comparisons with pretraining data.

performed to assess adaptation to intermittent hypoxia. The test determined the time it took SpO_2 to decline from the initial level to 80% during hypoxic gas breathing, with F_IO_2 =10% (Td SpO_2), and the time it took SpO_2 to recover from 80% to the initial level after the participants stopped inhaling the hypoxic gas (Tr SpO_2).

Statistical analysis was carried out in Mircosoft Excel 2016 (16.0.5071.1000) (Microsoft Corporation; USA). Normality of data distribution was tested using the Kolmogorov–Smirnov test. Significance of differences was assessed using Student's *t* test and the nonparametric Wilcoxon *T* test. Differences were considered significant at $p < 0.05$. The results are presented in the tables below as M ± *m*.

RESULTS

Mean SpO_2, HR, SBP and DBP measured during hypoxic gas breathing are provided in Table 1. Following exposure to the hypoxic gas mixture, SpO_2 decreased significantly by an average of 20.4% ($p < 0.05$), HR increased by 22% ($p < 0.05$) and DBP lowered by 4.5% ($p < 0.05$) relative to the initial levels. DBP did not change significantly. Subjectively, the participants tolerated the applied IHT protocol well and did not complain of any discomfort. SpO_2, HR and blood pressure went back to normal when the participants were breathing ambient air. The same dynamics repeated themselves over the next cycles throughout the session.

Table 2 shows changes in the hematological parameters of red blood cells and EPO during IHT. We observed a significant increase in the absolute reticulocyte count (16.6%; $p < 0.05$) following the completion of the 11-day IHT regimen. There was a distinct tendency toward elevated red blood cells and total hemoglobin ($p > 0.05$) in the setting of the increased reticulocyte count. At the same time, serum EPO concentrations declined by 44.2% ($p < 0.05$) relative to the initial values. In the second part of the experiment, the duration of IHT was extended to 14 days, which led to a significant 3.9% increase in red blood cells ($p < 0.05$) and a 4.7% increase in hemoglobin concentrations ($p < 0.05$) relative to the pretraining values. However, in contrast to the 11-day regimen, the absolute reticulocyte count was not elevated after 14 days of IHT. Moreover, the absolute reticulocyte count did not differ significantly from the initial level and was by 6.7% lower than at baseline ($p > 0.05$). On average, hematocrit concentrations were slightly above baseline values in both parts of the experiment. However, the changes were insignificant ($p > 0.05$).

Fig. 1 features the results of the functional test during hypoxic gas breathing (F_IO_2 =10%). After 14 days of IHT, the test showed a significant increase (by 93. 5%) in the time it took SpO_2 to lower to 80% ($p < 0.05$) and a statistically significant reduction by 44% ($p < 0.05$) in SpO_2 recovery time relative to the pretraining values. Considering the detected shifts in the hematological parameters of red blood cells, we hypothesize that these changes might be associated with the increased oxygen-carrying capacity of the blood following the IHT program and the developed adaptation in response to intermittent exposure to hypoxic hypoxia.

DISCUSSION

Normally, normobaric and hypobaric IHT regimens rely on P_IO_2 varying between 114 and 76 mmHg [7, 25, 26, 30–34]. In our study, P_IO_2 was maintained at 76 mmHg during the first training session but then adjusted to 68.5 mmHg for the remainder sessions. During the 14-day regimen, the participants did not have any health complaints or report discomfort. HR and blood pressure were within the normal reference range, suggesting that healthy men could tolerate the applied protocol well.

Table 1 demonstrates that the most pronounced changes in SpO_2 and HR were observed in the first part of week 1 of training. Starting from week 2, the decrease in SpO_2 became less pronounced, HR was growing more slowly, and DBP was significantly decreased. According to the literature, these changes might be associated with a relatively increased activity of the parasympathetic nervous system during adaptation to intermittent hypoxia [7, 8, 35] and with improved tolerance to hypoxia [36].

Our experiment demonstrates that changes in the hematological parameters of red blood cells become noticeable and statistically significant after 1.5 weeks of training. They encompass increased production of reticulocytes in the bone marrow and their mass release into the bloodstream. Today it is believed that elevated reticulocytes in the blood reflect the increased production of EPO, the major erythropoiesis regulator [37]. Under reduced P_IO_2, serum EPO concentrations peak in 24–48 h and can decline then a week later, approximating the initial level [38]. Erythropoiesis is a slowly activated process. Reticulocytosis becomes noticeable as late as 3–4 days after EPO elevation [37]. Our findings are consistent with the results of other studies investigating EPO dynamics. Low EPO levels and increased reticulocyte count detected after the completion of the 11-day regimen are in good agreement with the absence of reticulosis, significantly elevated red blood cells and increased hemoglobin after 14 days of IHT.

Apart from being the main physiological erythropoiesis regulator, EPO is involved in regulating the functions of the brain stem structures that control the respiratory system; specifically, EPO participates in the regulation of the hypoxic ventilatory response [39, 40]. A study measured the levels of EPO mRNA in the brain stem of rats following 2 weeks of intermittent hypoxic exposure at F_IO_2 equaling 12% or 7% [41]. The study found that EPO mRNA tended to decline following 2 weeks of moderately intense exposure to hypoxia (12% O_2) and dropped more than twofold after a more intense hypoxia regimen (7% O_2). The researchers linked the reduced EPO production to the completion of some adaptation stage after

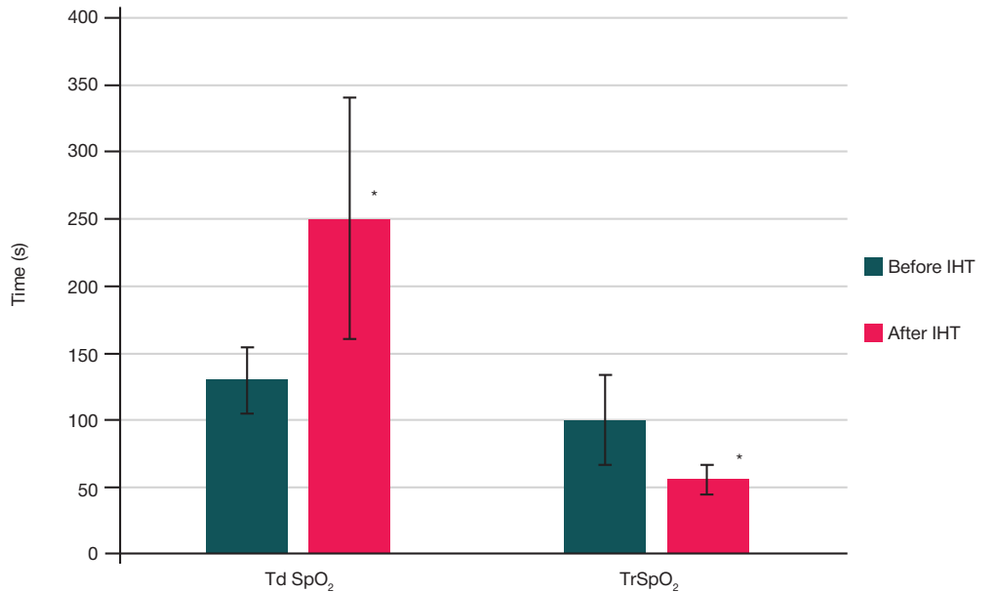

Fig. 1. $TdSpO_2$ and $TrSpO_2$ values during hypoxic gas beathing (F_iO_2 = 10%) before and after the 14-day IHT regimen. * — $p < 0.05$ for comparisons with pretraining values

IHT. However, it should be born in mind that EPO expression and the intensity of erythropoiesis are interrelated through O_2-dependent processes. There are reasons to assume that the initial elevation of serum EPO occurs when EPO production exceeds its utilization in the bone marrow, whereas EPO levels start to decline when increased erythropoiesis leads to the increased utilization of EPO in the bone marrow [42]. Thus, at each stage of adaptation to intermittent hypoxia a dynamic equilibrium will be maintained between the required level of EPO production in the kidneys and its utilization in the bone marrow.

The term “hypoxic dose” is often used in the academic literature about the hematological effects of hypobaric and normobaric IHT. It characterizes the capacity of an IHT protocol to have a sufficient stimulating effect on erythropoiesis by activating EPO production [26, 43]. This characteristic is determined by the PO_2 in the inspired air, the duration of hypoxic exposure in each cycle, periodicity of alternating exposures to inspired ambient and hypoxic air, the frequency of training sessions per week, and the total duration of the IHT program. We found that the applied 2-week regimen, which included 1-hour long daily sessions at PO_2 ~ 68.5 mmHg, was enough to activate erythropoiesis, increase red blood cell count, hemoglobin and oxygen-carrying capacity of the blood. With a relatively brief total exposure to a hypoxic environment, the applied hypoxic dose might not be sufficient to increase the total hemoglobin mass [32, 44].

In sports medicine, IHT has long been used to prepare athletes for competitions and improve oxygen uptake and physical performance [45]. However, in IHT the increased oxygen-carrying capacity of the blood is not the only contributor to better endurance performance [46]. Activated under reduced PO_2, HIF-1 was initially described as a transcription regulator for the EPO gene [47]. Later it was discovered that HIF-1 can activate a staggering variety of genes whose involvement is not limited to adaptive hematological responses [40]. HIF-1 plays a crucial role in the response of the cardiovascular and respiratory systems to hypoxia [48]. It initiates complex responses aimed at improving lung ventilation, angiogenesis, maintaining pH and acid-base metabolism in muscle tissue [46], improving oxygen uptake by cells [28]. Each of the listed non-hematological IHT effects can contribute to improving physical performance independent of the increased oxygen-carrying capacity of the blood.

CONCLUSION

The proposed regimen included 1-hour long normobaric IHT sessions at PO_2 ~ 68.5 mmHg and was administered to a group of healthy male volunteers. The regimen simple and well tolerated by the participants; it provoked moderate transitory changes in cardiorespiratory parameters. The 2-week IHT program based on the proposed regimen resulted in the increased red blood cell count and elevated hemoglobin, suggesting an improvement in the oxygen-carrying capacity of the blood. The proposed regimen can be used to improve physical performance of individuals working in extreme environmental conditions.

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DYNAMICS AND LOGIC OF COVID-19 CONTAINMENT MEASURES

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The ongoing COVID-19 pandemic has confronted public health systems and world economies with serious challenges. Faced with the same disease, countries responded to the threat differently depending on their social, demographic and geographic characteristics. Based on the analysis of scientific literature, international guidances and other sources of information about infection prevention and control, this article systematizes knowledge about containment strategies developed before the current pandemic, describes challenges posed by the coronavirus outbreak and highlights solutions. Specifically, the article describes the timing and order of the introduced measures, considerations for lifting the restrictions and the impact of different containment strategies on the spread of the infection, society and economy.

Keywords: COVID-19; pandemic; non-pharmaceutical public health measures; review

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ДИНАМИКА И ЛОГИКА ПРОТИВОЭПИДЕМИЧЕСКИХ МЕР

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Текущая эпидемия коронавирусной инфекции COVID-19 породила целый ряд вызовов для организации здравоохранения и экономики стран мира. Несмотря на то что все государства столкнулись с одним и тем же заболеванием, принимаемые экономические и организационные меры сдерживания его распространения заметно различаются в зависимости от их социальных, демографических и географических характеристик. Основываясь на аналитическом обзоре исследовательской и научной литературы, международных руководств и других источников, посвященных противоэпидемиологическим мерам, данная работа систематизирует знания о стратегиях сдерживания эпидемий, разработанных до текущей пандемии, и описывает вызовы, которые поставила перед миром вспышка нового коронавируса, и решения, принятые для ее предотвращения. В частности, показано, в какой последовательности и комбинации страны вводили разные меры, чем они руководствовались при отмене ограничений, а также то, как исследователи анализировали влияние разных стратегий борьбы с эпидемией не только на распространение инфекции, но и на социальные и экономические процессы.

Ключевые слова: COVID-19; пандемия, нефармацевтические меры общественного здравоохранения; обзор

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The first cases of the respiratory infection caused by the novel coronavirus SARS-CoV-2 were reported in Wuhan (South China) in December 2019. The infectious disease later termed COVID-19 (the acronym of coronavirus disease 2019) rapidly spread the globe. On March 11, WHO declared a pandemic [1]. The worst pandemics of the 20th century were caused by influenza viruses. With every new pandemic, researchers and public health experts learn more about the dynamics of such infections and refine measures for slowing their transmission and so reducing the number of new cases and deaths. Interventions that break the chain of virus transmission between humans are key in halting the spread of infection. They include identification and subsequent isolation of infectious individuals, contact tracing and quarantine of suspected cases and practices for reducing the risk of contracting the virus, such as good personal hygiene and social distancing in the first place. During the current pandemic, governments took unprecedented nationwide measures to prevent healthcare capacities from overwhelming and curb the risks of infection. Unfortunately, those containment measures came at a cost: they caused tremendous damage to economies, public welfare, health and psychological wellbeing. At the outset, the decisions made were based on the experience of past pandemics. However, over time more and more new, long-term restrictions were imposed; some of them were perceived as extreme, with unjustifiably high costs for the society. Today, as many countries are preparing to reintroduce strict anti-COVID measures, it is time the experience of world governments was summarized and relaxation strategies were discussed.

Containment measures and their types

Strategies for countering epidemics aim at slowing the transmission of the virus, suppressing and preventing its outbreaks. Actions that can be taken by individuals, communities, organizations, and governments to prevent or slow the spread of infection can be broken down into a few categories [2–4]:

– surveillance and rapid response to identify and isolate infectious individuals, trace and quarantine their contacts;

– personal protective measures (good hand hygiene,

physical distancing, respiratory etiquette, wearing face masks that cover the mouth and nose);

– environmental measures (surface and object cleaning, using UV light, improving ventilation and adjusting air humidity);

– physical and social distancing in public spaces (physical distancing, limitations on mass gatherings or their cancellation, avoiding crowds on public transport, in restaurants, theaters or shops, school closures and distance learning, working from home, restrictions on visiting public spaces);

– travel restrictions to prevent the spread of the virus to other regions (travel advice, planning trips in advance to avoid congestion at railway stations, bus terminals and airports, restricting or banning region- or nationwide trips);

– special measures can be imposed to protect certain groups of population: those at risk for developing severe infection, individuals in institutional care (care homes, prisons, etc.), or those occupationally exposed to the virus.

There are other measures that are not directly associated with halting the transmission of the virus but that can make a significant contribution to fighting the epidemic [5]. For example, governments can:

– establish /summon emergency management agencies and declare a state emergency;

– invest funds in the research and development of vaccines and treatments;

– strengthen public health systems, i.e. institute measures for improving public health funding, satisfying the need for hospital supplies and equipment, reshaping work environments for healthcare workers and other specialists;

– expand the arsenal of social relief tools that minimize the negative impact of the imposed restrictions on the socioeconomic activity of the population, including measures to support economy, financial aid to individuals and federal agencies.

Thus, governments have a broad armamentarium of strategies to reduce contact rates between people and curb the transmission of the virus. If successful, these interventions curtail the epidemic and spread the number of infected cases over time, preventing public health capacities from overburdening. However, prior to deciding on the type, timing and intensity of containment measures, their effectiveness should be thoroughly analyzed, which may be a challenge due to a possible lack of information about the novel pathogen, as was the case with SARS-CoV-2.

The effectiveness of containment measures is determined by many variables, from demographic to geographic. Poor compliance remains a problem. The decision to self-isolate and keep social distance is largely determined by income and employment type. Residents of high-income countries with sustainable social welfare policies, as well as affluent citizens, are at lower risk of losing their source of income during an epidemic and have better chances to cope should this risk occur.

Lastly, when imposing containment measures, governments should not ignore their "side effects", i.e. social implications and economic costs.

What did we know about the efficacy of containment measures before COVID-19?

Previous pandemics of respiratory infections were caused by influenza viruses. The pandemic triggered by Spanish flu (virus A (H1N1)) in 1918–1919 was the largest: it is estimated to have killed 20–50 million people. Smaller pandemics occurred in 1957–1958 (Asian flu, virus A (H2N2)), in 1968 (Hong-Kong flu, virus A (H3N2), with 1-4 million fatalities each, and in 2009–2010 (virus A (H1N1), with the death toll of 100,000–400,000 [6, 7]. The 21st century has already witnessed 2 coronavirus epidemics of SARS in 2002 and MERS in 2012, but neither of them spread globally. SARS infected about 8,000 and killed 800 people, whereas MERS, 2,500 and 850 people, respectively [8].

Measures for containing the spread of COVID-19 were largely based on the information obtained during those epidemics.

In 2019, WHO released a systematic review of non-pharmaceutical public health measures for mitigating the risk and impact of endemic and pandemic influenza [2]. This meta-analysis focused on the effectiveness of non-pharmaceutical interventions using data from MEDLINE, PubMed, EMBASE, Cochrane library and Cochrane Central Register of Controlled Trials. Final recommendations accounted for the level of evidence, weighted benefits against costs of implementation, assessed feasibility of the interventions and the resources needed (Table. 1).

Unfortunately, for some interventions the quality and amount of evidence are insufficient to conclude that the intervention should or should not be implemented during an influenza pandemic. For example, in contrast to UV light that has been proved ineffective, the effectiveness of border closure is debatable due to the dearth of data. Studies addressing the effects of containment measures during SARS and MERS epidemics are even scarcer. In 2015, WHO released a *Guidance for infection prevention and control during health care for probable or confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV)*, which was updated in 2019 [10]. According to the Guidance, *"human-to-human transmission occurs mostly in health-care settings and, to a more limited extent, within communities, mainly in households… Further research is needed to understand the risk factors for viral transmission from animals to humans and between humans"*. The Guidance thus focused on healthcare provision for infected individuals in inpatient facilities; no recommendations were proposed for outpatients, communities and governments.

Of note, the Guidance does not list contact tracing and quarantine for exposed individuals because these measures are ineffective in case of influenza. The fact that COVID-19 can be asymptomatic and that asymptomatic cases contribute significantly to its spread was established later. An infected person appears to be able to transmit the virus 2–3 day before the onset of symptoms, suggesting that contact tracing and quarantine of exposed individuals is a very effective containment measure [11, 12].

As a personal protective measure, wearing gloves was strongly recommended and even was mandatory in some Russian regions. But gloves are not mentioned in WHO guidelines as a measure to contain the spread of influenza [2] or COVID-19 [4]. Moreover, there is evidence that health damage provoked by wearing gloves outweighs the benefits [13].

Open-access data for analysis of COVID-19 containment measures

Governments across the world took unprecedented action to contain the spread of COVID-19. From the outset of the pandemic, researchers have been monitoring the measures taken and collecting valuable data that can now be used to develop effective strategies against the virus. Below, we provide a few examples of such collections.

Table 1. Recommendations on introducing non-pharmaceutical interventions according to the severity of epidemic or pandemic flu (adapted from [2])

Severity*	Pandemic	Epidemic
Any	Hand hygiene Respiratory etiquette Face masks for symptomatic individuals Surface and object disinfection Increased ventilation Isolation of sick individuals Travel advice	Hand hygiene Respiratory etiquette Face masks for symptomatic individuals Surface and object disinfection Increased ventilation Isolation of sick individuals Travel advice
Moderate	*As above plus* Avoiding crowding	*As above plus* Avoiding crowding
High	*As above plus* Face masks for everyone School measures and school closures	*As above plus* Face masks for everyone School measures and school closures
Extraordinary	*As above plus* Workplace measures, workplace closures Internal travel restrictions	*As above plus* Workplace measures, workplace closures
Not recommended	UV light Modifying air humidity Contact tracing Quarantine of exposed individuals Entry and exit screening Border closure	UV light Modifying air humidity Contact tracing Quarantine of exposed individuals Entry and exit screening Internal travel restrictions Border closure

Note: * — Pandemic influenza severity assessment (PISA) was based on the transmissibility of the virus, severity of the disease and its impact on public health and society. Five levels are distinguished: no activity/activity below seasonal threshold, low, moderate, high, and extraordinary activity [9] (based on [2]).

WHO Public health and social measures (WHO PHSM)

The database [14] comprises data aggregated from different credible sources and classified into the following categories:

– biological measures;
– drug-based measures;
– environmental measures;
– individual measures;
– international travel measures;
– other measures;
– social and physical distancing measures.

The first two classes are closely linked to the trialing of drugs, vaccines, etc. (these categories are rarely included in other datasets describing measures against COVID-19). The “Other measures” class refers to all economic measures initiated by governments, e.g. working from home.

Example: on March 22, the government of Germany banned gatherings of more than 2 people: 2 people could meet up if they kept physical distance of at least 1.5 m. According to the classification scheme listed above, this measure falls under the “Social and physical distancing measures” class, the “Gatherings, businesses and services” subclass and the “Cancelling, closing, restricting or adapting public gatherings outside the home” action.

At the time of writing, there was no information about the timing of the implemented measure although the column was present in the classification table.

COVID19 Government Measures Dataset

This database was created under the non-profit non-governmental international ACAPS project [15]. Categories:

– social distancing;
– movement restrictions;
– public health measures;
– social and economic measures;
– lockdowns.

Example: the German ban on gatherings of more than 2 people falls under the “Social distancing” category, the “Limit public gatherings” measure and is described as “Limit to the number of people that can meet in public and private spaces.”

The Oxford COVID-19 Government Response Tracker (OxCGRT)

It is probably the most consulted source that provides information about government response to epidemics and proposes a few indicators for quantitative analysis, which considerably simplifies inter-country comparisons [16].

OxCGRT accumulates information on containment measures implemented by world governments and gauges government response using 17 indicators. Of them, 8 refer to virus containment (school closure, travel restriction). Five indicators reflect public health policies (testing, emergency investment in public health). Four indicators characterize economic policies (income support).

Based on these indicators, 4 indices have been developed, each of them being a number between 0 to 100 [17]: 1) the overall government response index sums up all government actions for each indicator type, showing how the government response transformed over time, becoming stronger or weaker during the outbreak; 2) the stringency index reflects the stringency of the restrictions (imposed on the population in the first place), including lockdowns, restrictions on travel, mass gatherings, and social distancing; 3) the containment and health index evaluates a combination of stringent policies and public health measures (testing, contact tracing, investment into vaccine development, etc.); 4) the economic support index.

With indices that simplify quantitative analysis, accuracy will be inevitably sacrificed for convenience. For example, restrictions on mass gatherings are classified using the following scale:

0 — no restrictions;
1 — restrictions on very large gatherings (over 1,000 people);
2 — restrictions on gatherings between 101 and 1,000 people;
3 — restrictions on gatherings between 11 and 100 people;
4 — restrictions on gatherings of 10 people or less.

Thus, restrictions on mass gatherings for 2, 5 and 10 people score the same on the proposed scale.

The scale for school and university closures seems to be even rougher:

0 — no measures;

1 — recommend closing;

2 — require closing (only some levels or categories);

3 — require closing all levels.

In some countries, long-term closures were forced on all educational institutions. In others, universities and schools were not closed simultaneously, or shutdowns were mandated for some levels only (primary schools), or schools remained open only for the children of residents involved in essential continuous production cycle enterprises. Some nuances were lost while evaluating the stringency of the implemented measures. Summing up, indices are simple and effective tools for comparing containment measures taken by the governments of different countries. To analyze an individual country, desegregated indices of its policies should be used.

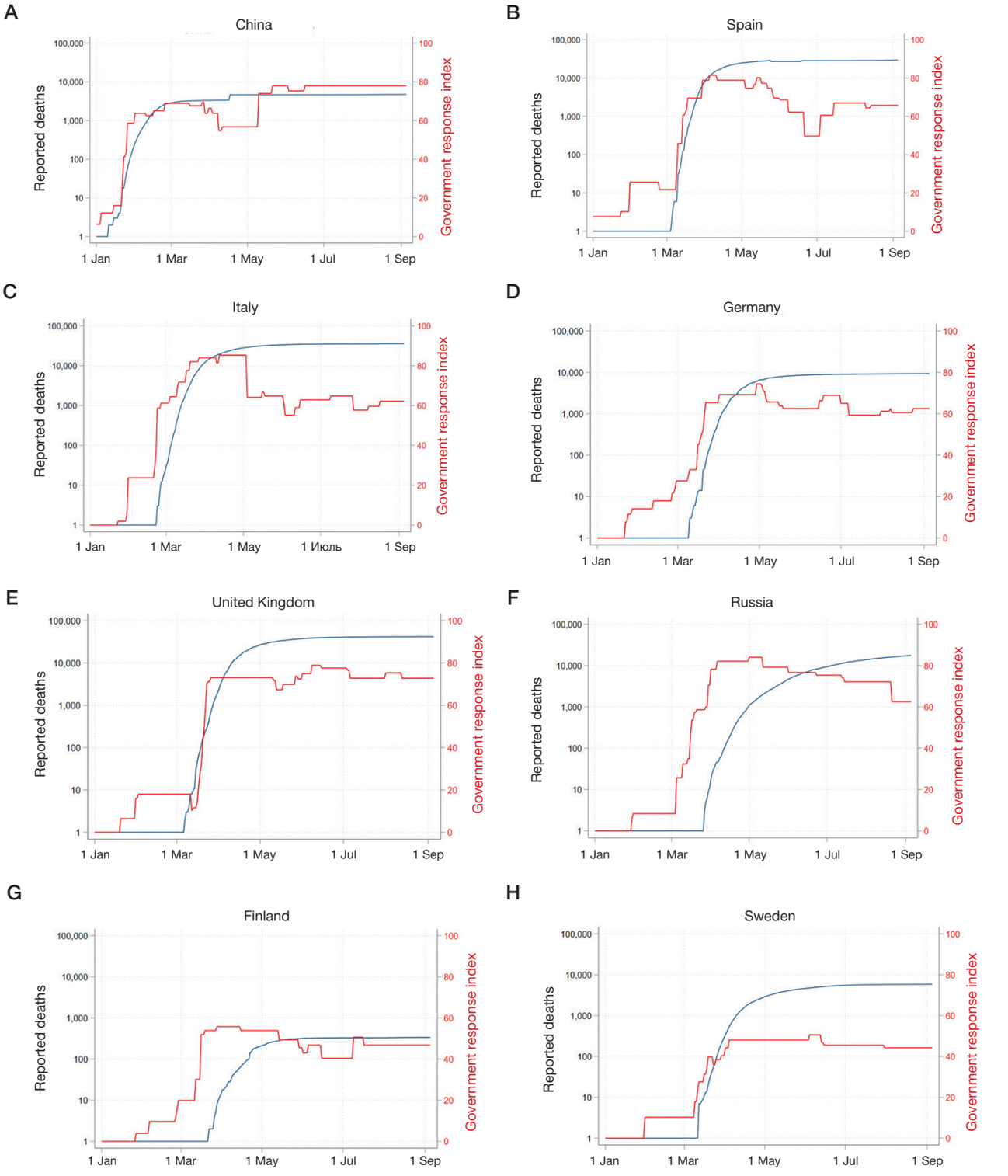

Fig. 1. The dynamics of the overall government response index proposed by OxCGRT and the number of confirmed deaths in 8 countries. The reported number of deaths is plotted on the left axis; the government response index is plotted on the right axis; its curve almost repeats the shape of the curve for the number of confirmed cases [16]

When and how did countries introduce containment measures?

At the outset of the pandemic, governments had to rely on the recommendations based on the experience of past epidemics and pandemics and navigate in uncertainty as there was no information about the novel virus and the disease it caused. The severity of an epidemic depends on the transmissibility of the virus (see Table 1), which back then was unknown. It was impossible to determine the number of infectious individuals and difficult to count all the sick. The main routes of transmission were only hypothetical, no information was available about the early symptoms and the course of the disease; its incubation period was uncertain. It was not clear how big a gathering had to be to be banned: over 1,000 people? Over 500? Over 50? Should people not congregate in groups over 3? Since the start of the pandemic, even the general public has become accustomed to the term "effective reproductive number", understood the difference between lethality and mortality rates, and started to realize that governments took decisions based on the available information.

Fig. 1A–H illustrate the dynamics of the overall government response index (OxCGRT) and the number of deaths from COVID-19 in 8 countries. Shortly after the initial outbreak in China, it became clear that the virus was spreading at a sweeping pace and its impact on public health systems would be immense. Severe patients required a complex lengthy and resource-consuming treatment. It was estimated that healthcare capacity, which takes time to increase, would be overwhelmed if the rate of spread and the death toll would continue to grow at the same pace. So, governments hurried to take large-scale action. In late April and early May, Spain and Italy urgently introduced harsh measures to control the spread of the virus. The measures (the red line in the figure) were triggered in the wake of the exponential growth of confirmed COVID-19 cases (not shown in the figure) and the soaring number of deaths (the blue line). In the UK, Boris Johnson's government faced a barrage of criticism for delaying the introduction of stringent containment measures. In Germany, the government response followed the trajectory of confirmed cases and was slightly ahead of the death curve. In Russia, strict measures were somewhat preemptive, drawing on the experience of Western countries. In Finland, interventions were more stringent and urgent than in Sweden, but on the whole the stringency index for Nordic countries was more than 20 points lower than in Spain, Italy, and Russia.

Protracted stringent measures, specifically quarantine, may have a disincentive effect: over time, people (and society in general) grow reluctant to comply with the restrictions [18]. A review by a team of medical psychologists provides evidence of negative psychological effects exerted by quarantine [19]. Self-isolation and lockdowns lead to post-traumatic stress, depression, and anger that last long after the restrictions are lifted (up to 3 years); there is also evidence that voluntary self-isolation is better tolerated than mandatory [19].

Perhaps, Sweden took heed of those warnings. When the pandemic started, Sweden was harshly criticized for its weak policies. However, maybe it won strategically, averted an economic recession and did not disincentivize the population to comply with the restrictions. As a result, the Swedish population is likely to be far more cooperative with their government during the second and subsequent epidemic waves than populations of other European countries. It is speculated that the UK government was trying to delay stringent measures in an effort to find the right time when lockdown benefits outweighed its costs, so that the fatigue felt by the population would not disrupt the positive effects of quarantine.

When and why were strict measures relaxed?

Containment measures were relaxed (or maintained, as in China, UK and Finland) when the number of deaths reached the plateau (see Fig. 1). Russia is an exception here because it eased the measures at the time when death rates were growing. Relaxation and reintroduction of containment measures is a stepwise process that largely depends on the number of confirmed cases and fatalities, as well as new information about transmission routes. The epidemiological situation in the region determines the order in which containment measures will be lifted. Agencies responsible for infection prevention and control and regional governors estimate the number of sick individuals and compare it against healthcare capacities in order to prevent the public health system from overwhelming. WHO suggests that at least 6 criteria should be accounted for when deciding on the timing for lifting containment measures [20].

1. COVID-19 spread is confirmed to be under control.
2. Public health system capacities are sufficient for timely identification, isolation, testing, contact tracing and quarantine.
3. Vulnerable populations are protected: risks of outbreaks in care homes and psychiatric facilities have been minimized; the same pertains to mass gatherings.
4. Measures for COVID-19 prevention in the workplace are strictly adhered to, including social distancing, good hand hygiene and respiratory etiquette.
5. Risks of "importing" the infection from other regions can be adequately managed.
6. The public is aware of the situation and ready to cooperate.

Another factor that affects the order in which measures may be relaxed is local culture, including compliance of the population with the recommendations and restrictions, significance of social contacts or activities in the particular cultural setting. Many countries develop response frameworks that allow for some variation across different regions depending on the local culture, which determines the priority of public places that should open first and their working hours, the need to self-isolate for people from other regions, the stringency of restrictions on mass gatherings, etc. Many countries are also developing long-term lifestyle and work model, i.e. rules that will be perceived as a new normal until the virus is no longer a threat.

So far, general recommendations regarding physical distancing, hand hygiene, respiratory etiquette and wearing face masks in certain settings remain in force in most countries affected by COVID 19. However, restrictions can be mitigated or toughened at any time, and anti-COVID policies are updated almost every week. For instance, it was only in mid-August that ban on marriage ceremonies (with no more than 30 guests present) was lifted in the UK and spas and some other small businesses opened; in some UK regions in-home mass gatherings of over 10 persons are still prohibited . Russia relaxed some of the strict measures in June and July although the virus had spread to our country later than to most European countries; this may be explained to regional differences. The measures that are still in force in Russia include wearing face masks in public places and thorough disinfection. At the same time, Finland, which was the first to open schools and did not have a face mask mandate in the spring of 2020, issued a recommendation for the public in mid-August on wearing face masks on public transport; this decision may be regarded as an introduction of new measures for preventing the spread of the coronavirus infection.

Assessing effectiveness of containment measures and their impact on economy

Decisions on instituting containment measures and assessment of their effectiveness at different stages of the pandemic require robust, reliable, up-to-date data on the infection itself and the mobility, behavior and compliance of the population. Understanding the dynamics of population mobility and population response to the introduced interventions will help to 1) predict the geographic spread of the disease and thus estimate future risks, demands and implementation potential, and 2) identify causal links and mechanisms and assess the contribution of each measure, which may improve the effect of their implementation [21]. Such data can be acquired through different routes.

Surveys are a traditional tool for collecting data. They are useful in tracing social contacts, estimating the impact of the introduced interventions on income and employment, and measuring public support. A survey was launched in the UK a day after the lockdown started [22]. The survey was conducted in a representative sample of adults. Respondents were asked about contacts they had had on the previous day and report the events they had planned to visit during the preceding week but had to cancel. Respondents were asked about their adherence to social distancing requirements during thew preceding week. Respondents provided information on members of their households who had been recommended to self-isolate or limit their time at work or at an educational institution. They were also asked whether they had reduced the number of social contacts voluntarily and if so, how. Thus, the researchers created models and compared the number of social contacts before and during the lockdown. Then they analyzed changes in R0 following the introduction of physical distancing measures. It was found that the average daily number of contacts per participants decreased by 74% (from 10.8 to 2.8) during the lockdown. This was enough for R0 to fall from the pre-lockdown value of 2.6 to 0.62 (95% CI: 0.37–0.89) for all types of contacts during the lockdown and to 0.37 (95% CI: 0.22–0.53) for skin-to-skin contacts.

Digital data, including data from mobile phones, are an important analytical tool as they help to monitor the dynamics of population mobility in almost real time and therefore are very useful in predicting the spread of infection and the effectiveness of measures taken [23]. A good example is data from [24]. The study sought to understand the effect of measures implemented by state and local governments (emergency declarations, school closures, rules for restaurants, restrictions on mass gatherings, business closures, stay-at-home mandates) on social distancing at the outset of the epidemic in the USA. The researchers analyzed geolocation data from mobile apps collected by private companies. The data included information about the number of mobile phones simultaneously present at a location visited by the owner of the tracked mobile device during the day; about the time spent by the owner at home and outdoors; about the relocation of the device across the state and to other states. Considering that measures taken by different states were not introduced simultaneously and varied in intensity, the authors of the study concluded that adequate information and recommendations were as effective in reducing mobility as enforced social distancing measures.

Instantaneous contact tracing by means of a mobile application and subsequent automated notification of close contacts may be sufficient to halt the epidemic if the app is used by a high proportion of the population [26]. Supported by the European Commission, the eHealth Network initiative developed a set of tools for creating and using contact tracing apps compliant with the EU principles of confidentiality and data protection [27].

Epidemiological models are another tool widely exploited to assess the effectiveness of containment measures. Using examples from the literature, the authors of the study [28] developed a SEIR model to simulate measures for infection prevention and control varying in duration and intensity for one year. The study demonstrates that physical distancing measures should be lifted gradually in order to avoid peak incidence and prevent public health systems from collapsing.

More complex epidemiological–economic models account for individual behaviors in response to the threat of infection [29]. Studies demonstrate the effectiveness of aggressive containment policies and early, stringent social distancing measures aimed to reduce death rates and mitigate economic costs [30–33]. New models for analyzing the effectiveness of public health measures are underway. For example, a Bayesian model was developed that estimates transmission from observed deaths and simulates a hypothetical counterfactual scenario to estimate the number of deaths that would have occurred if containment measures had not been introduced [34]. According to the study, the introduced public health interventions led to a drop in Rt below 1 and thus helped to avoid 3,100,000 deaths in 11 European countries.

Some studies emphasize that testing for COVID-19 and the subsequent isolation of infected individuals reduces the need for stringent social distancing measures and thus allows finding a tradeoff between low economic activity and public health [35–37].

A multi-risk SIR model (MR-SIR) in which the rates of infection, hospitalization and fatality varied between different age groups (young, middle-aged and old) showed that optimal measures differentially targeting risk/age groups worked significantly better than “one-size-fits-all” measures targeting the entire population; the analysis revealed that at the same level of economic damage greater gains (in terms of fatality reduction) could be achieved if stricter isolation policies were applied to the oldest group [38]

Importantly, working from home may not be an option for every sector of the economy; this should be accounted for when lifting the restrictions. A broad “reopening” of the economy is still possible if stringent restrictions are imposed on social contacts outside work (mass social gatherings, attending restaurants, bars, etc.) [39, 40].

CONCLUSION

Despite the rapidly growing number of studies addressing the effectiveness of public health and social measures and their implications for the economy and society, the accumulated data are still insufficient to draw firm conclusions about their relevance and adequate timing. The scope and stringency of measures introduced to contain the spread of COVID-19 were unprecedented. Some of them (contact tracing, restrictions on international travel, physical distancing) were tested and applied for the first time in history.

The order, timing and the scope of public health and social measures depends on the social, demographic and geographic characteristics of a country. Besides, success in curtailing the epidemic is to some extent determined by the experience the country had with other infections, its healthcare capacities and economic development. Importantly, the effectiveness and consequences of containment measures can vary across different social groups within the same country: during

the ongoing COVID-19 pandemic, elderly and low-income populations turned out to be the most vulnerable.

Differences in the stringency and timing of containment measures between countries can be analyzed using databases that gauge government responses using a set of indicators and indices. Such indices simplify data comparison but have certain limitations because they provide very rough estimates for individual cases or regions. Disaggregated data should be used to assess the effectiveness of containment measure within a given country. Besides, as the pandemic is continuing, the data are being accumulated and databases are being upgraded, so the effects of containment measures on the economy, social and political institutions are yet to be elucidated.

Although containment strategies turned out to be quite effective and significantly slowed or halted the spread of the novel coronavirus in some countries, the society and the world economy are still facing challenges posed by the pandemic and therefore have to develop new interventions to counter the threat.

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COVID-19 IN OPHTHALMIC PRACTICE

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The end of 2019 in China was marked by the breakout of the new Coronavirus Disease (COVID-19) caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Gradually, the infection spread around the world and in March 2020, the World Health Organization (WHO) declared Covid-19 a pandemic. The new coronavirus disease 2019 is highly contagious, causing respiratory distress syndrome and poses a huge threat to public health, especially in patients. with serious concomitant diseases such as diabetes mellitus, bronchial asthma, hypertension, etc. Many scientists have put forward the idea that COVID-19 can be transmitted through the eyes through contact and everyday life. Over the past six months, works on the ocular manifestations of coronavirus infection have begun to appear in the literature. We conducted a systematic review of scientific articles from the PubMed, e-Library, Scopus databases in order to conduct a meta-analysis of the effect of coronavirus infection on the eyes and its ophthalmological manifestations.

Keywords: coronavirus infection, COVID-19, coronavirus, coronavirus conjunctivitis

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COVID-19 В ОФТАЛЬМОЛОГИЧЕСКОЙ ПРАКТИКЕ

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Конец 2019 г. в китайском городе Ухань был отмечен вспышкой новой коронавирусной болезни (COVID-19), вызванной SARS-CoV-2. Постепенно инфекция распространилась по всему миру и уже в марте 2020 г. Всемирная организация здравоохранения объявила COVID-19 пандемией. Новая болезнь высококонтагиозна, вызывает респираторный дистресс-синдром и представляет собой огромную угрозу для здоровья населения, особенно у пациентов с серьезными сопутствующими заболеваниями, такими как сахарный диабет, бронхиальная астма, гипертоническая болезнь и др. Выдвинуты предположения, что COVID-19 может передаваться через глаза контактно-бытовым путем. За последние полгода в литературе стали появляться работы, посвященные глазным проявлениям коронавирусной инфекции. На основании обзора научных статей базы данных PubMed, e-Library, Scopus проведен метаанализ влияния коронавирусной инфекции на глаза и ее офтальмологических проявлений.

Ключевые слова: коронавирусная инфекция, COVID-19, коронавирус, коронавирусный конъюнктивит

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Coronaviruses are enveloped RNA viruses of the Coronaviridae family. They contain four main structural proteins: spike protein (S-protein), nucleocapsid, membrane and envelope proteins. There is a lipid membrane around the capsid, which contains the proteins. As seen with an electronic microscope, the structure of the virus resembles a crown, hence the name. Nucleocapsid, membrane and envelope proteins mainly contribute to formation and structuring of the virus, while spike protein enables binding to host cells [1–3]. In human beings, these viruses cause respiratory tract infections, their symptoms being nasal congestion, rhinorrhea, sore throat, fever, cough, fatigue, muscle pain. The less common symptoms are diarrhea, tachycardia, headaches, chills, anorexia. In most cases, COVID-19 takes a mild form, but with cardiovascular diseases or immunosuppressive conditions in the background, the case can become severe and aggravated with respiratory failure. There are also reports of patients that tested positive for SARS-CoV-2 and had the subsequent disease running fully asymptomatic. Such patients can also be a source of infection [2–5].

Primarily, the virus is transmitted between people via airborne and contact routes. Receptors of angiotensin-converting enzyme 2 (ACE2), to which the virus's S-protein binds, enable infection of the cells. ACE2 receptors can be found in vascular endothelium, smooth muscles of the arteries, small intestine, respiratory tract epithelium, alveolar monocytes and macrophages. The contact route lies through the MERS-CoV (Middle East COVID-19 infection) receptor — DPP4 (dipeptidyl peptidase). DPP4 receptors are found in the respiratory tract epithelium, kidneys, small intestine, liver, prostate gland, and activated leukocytes [1–4].

While COVID-19 is primarily a viral pneumonia, in some patients SARS-CoV-2 caused eye disorders [1, 2, 6, 7]. Unfortunately, there is not much data on the effects COVID-19 has on the eyes. Following the spread of the infection, only a few reviews and clinical observation reports were published that covered coronaviruses from the ophthalmological perspective [4, 6–12].

Some researchers believe that SARS-CoV-2 may spread through mucous membranes, including the conjunctiva, in addition to the airborne and contact routes [2].

There is a well-known case of SARS-CoV-2 infection that exemplifies the point: a member of the National Group of

SARS-CoV-2 Experts got infected while wearing a protective suit and a mask but no glasses to protect the eyes. A few days before his pneumonia developed, he complained of red eyes. Thus, it can be assumed that the virus got in through the unprotected eyes.

Another case report describes a 65-year-old diabetic man who initially had eye-lesion and only two days after his first complaint developed a fever. This patient tested positive for SARS-CoV-2 (nasopharyngeal swab and PCR test). The authors concluded that all cases of keratoconjunctivitis concomitant with the upper respiratory tract disorder symptoms should be considered possible cases of COVID-19. Since virus RNA was found in the conjunctiva, many researchers deduced that the disease can be transmitted through the eyes [3, 13].

Hypotheses about how the virus lands on the ocular surface

Virus landing directly on the conjunctiva

Most researchers share the opinion that the virus infects the eyes in case infected droplets land on the conjunctiva directly. The studies of great interest are those designed to detect SARS-CoV-2 in the conjunctival secretions of the novel coronavirus pneumonia patients with the help of reverse transcription polymerase chain reaction (RT-PCR) [9]. There is a recorded case of SARS-CoV-2 RNA detection in a two-day conjunctival smear taken from a keratoconjunctivitis patient in Italy. In another case, SARS-CoV-2 was grown in an ocular smear taken from a patient that had been experiencing symptoms of the infection for three days. There are oppositely different cases described, too, when no virus RNA was detected in the lacrimal fluid of an inpatient with conjunctival infection and chemosis but, with respiratory symptoms in the background, that patient's nasopharyngeal smear returned SARS-CoV-2 positive [10, 12].

There are reported cases of detection of the virus in the lacrimal fluid. However, not all studies have confirmed presence of the virus in SARS-CoV-2 patients' tears and conjunctiva scrapings with a PCR test. The lack of such confirmation may be explained by insufficient sensitivity of the test, testing outside of the positive time window eye tissue immunity to SARS-CoV [10, 11].

Virus contraction through the nasolacrimal duct

When the patient has it in the upper respiratory tract, the virus can travel through the nasolacrimal duct and infect the eyes. This hypothesis stems from the case of an ER nurse that worked with SARS-CoV-2 patients. On the first day of the illness, her eyes were excessively red and tear shedding, so she was admitted to the ophthalmological department. No other systemic symptoms were reported except for the moderate temperature of 38.2 °C. Bacterial, hemorrhagic and allergic varieties of conjunctivitis were excluded. The nurse worked in a protective suit, glasses and a medical respirator, but she noted that the glasses did not fit tightly, constantly moved and touched the eyelids with their edges. Chest CT revealed multiple ground-glass opacities in the lungs. Conjunctival and oropharyngeal smears tested for SARS-CoV-2 returned positive results. Based on the epidemiological characteristics, clinical manifestations, chest images, the patient was diagnosed with acute viral conjunctivitis, SARS-CoV-2 infection, and pneumonia. However, there are also opposite cases. In China, conjunctiva biological material and lacrimal fluid were collected from patients having no ocular manifestations of the disease (or any such symptoms) within three weeks after infection. The subsequent examination detected no viral RNA even in samples taken from the patients showing symptoms of an upper respiratory tract infection. The authors of this study concluded that the hypothesis posing tear duct as a virus transmission channel may be questionable and requires further research [13].

Virus exudating from the vessels

There is another route the virus can take to infect the eye. Researchers have reported exudation from the vessels as a path forward for the infection, having discovered that SARS-CoV-2 invades endothelial layer of blood vessels. This, in turn, leads to disruption of blood microcirculation in organs and disruption of their functions.

Examination of the histological material of vessels revealed that COVID-19 patients have walls of their blood vessels showing signs of inflammation. It has been suggested that SARS-CoV-2 triggers a systemic inflammation of blood vessels that can affect heart, brain, lungs, kidneys, and eyes, causing severe microvascular disorders with organ dysfunction. The ACE2 receptor, to which the virus binds with the S-protein, is actively expressed in capillary pericytes. Results of the research efforts have shown that a reduced number of pericytes makes microvascular endothelial cells produce and release blood plasma glycoprotein more actively, this protein enabling platelet attachment to the damaged part of the vessel, which can explain the increased thrombosis development rate. The authors emphasize the fact that their hypothesis is a preliminary one and requires further confirmation [6–10].

Clinical manifestations of eye infection

The clinical manifestations of damage to the eye are diverse. The virus can affect both the anterior and the posterior segments of the eye. According to the published reports, the most frequent complaints are eye redness, itching, blurred vision and tear shedding. As noted above, the infection may spread via ACE2, which makes it interesting to note that epithelial cells of cornea and conjunctiva were found to express ACE2. S240, an isolated surface protein of coronaviruses, can bind to epithelial and fibroblast cells of conjunctiva and cornea epithelial cells, ACE2 enables binding on the cell surface. There is another receptor, CD209, found on the dendritic cells of human cornea and participating in transmission of the infection [3].

Frequently, the eye-related manifestations of the disease at its initial stage take form of conjunctivitis. There are many clinical cases of coronavirus-induced conjunctivitis reported in the published papers. For example, there is a coronavirus conjunctivitis case of a 65-year-old woman who returned to Italy from the city of Wuhan in China. She was admitted to the hospital one day after COVID-19 symptoms manifested. One of those symptoms was bilateral conjunctivitis, which persisted for 16 days. The conjunctival scrapings returned positive for viral RNA for 21 days after admittance.

According to a study on cats, in addition to conjunctivitis, initial stage infection can take the form of anterior uveitis, choroiditis with retinal detachment, neuritis and retinal vasculitis [4, 14, 15].

Numerous reports indicate that vascular changes and thrombotic events, including ischemic brain damage, are among the main complications brought by COVID-19. Based on the aforesaid, there is an assumption that the retina may also be involved in the pathological process [12–15].

Effect of SARS-CoV-2 on the retina

There is little data on the effect SARS-CoV-2 has on the retina. ACE2 virus entry receptors have been found in the retina of rodents and pigs. Ocular tissue of the latter had ACE2 in the ciliary body, vitreous and retina. Rodents' retina had ACE2 expressed in the inner nuclear layer, mainly in Müller's cells [10]. In human beings, ACE2 receptors have also been found in aqueous humor [14–16]. Researchers agree that SARS-CoV-2 can also infect the retina [4].

Among the published materials, there are studies aimed at searching for the virus RNA in the human retina. For example, German scientists have found RNA of the virus in 3 retina samples out of 14 taken from confirmed COVID-19 victims. In that experiment, retinal detachment was induced in order to prevent mixing of the sampled biopsy material with choroidal structures, since blood is another source that can spread the virus [4].

Researchers from Spain reported results of a study of retinal changes in COVID-19 patients. Microangiopathy was found in 22% of patients; it took the form of clusters of velvet spots [16, 17].

Still, it is an open question whether retinal microangiopathy in COVID-19 patients is brought by the virus immediately or if it is a manifestation of other systemic vascular diseases [17, 18]. The damage mechanism requires further investigation. It is interesting to note that ACE2 is the main enzyme of the vasoprotective renin-angiotensin system, and diabetic retinopathy is associated with an imbalance between the renin and the angiotensin-aldosterone system of the retina [16]. A decrease in the ACE2 level may play an important role in triggering development of retinal ischemia and even signal of endothelial dysfunction. There are at least two types of microvascular damage to the retina of COVID-19 patients: first, due to hypercoagulability, a disseminated intravascular coagulation syndrome [19]; second, through a process similar to vasculitis, which is the result of direct viral effect on endothelial cells and diffusive endothelial inflammation. However, despite the fact that patients received heparin, 22% of them, as mentioned above, had microangiopathy. The authors suggested that ophthalmoscopic examination may help identify patients with signs of arterial microangiopathy for whom antiaggregation may be of therapeutic importance [17–19].

Similar changes in the retina, namely vasculitis, were found in children. When examining fundus, authors of one of the studies observed changes in the vessels at the equator of the left eye, as well as perivascular infiltrates and dilated retinal exudates [20].

With the help of optical coherence tomography, some researchers assessed retinal changes in COVID-19 patients and people who recovered from the disease [21]. The patients were examined 11 to 33 days after the onset of COVID-19 symptoms. Two different OCT machines were used: DRI-OCT TritonSweptSource (Topcon; Japan) and XR Avanti SD-OCT (Optovue; California, USA). Every patient examined had normal visual acuity and pupillary reflexes; there were no signs of intraocular inflammation detected. In some patients, fundus ophthalmoscopy also revealed vascular changes, such as velvet spots (infarctions of the retinal nerve fiber layer) and microhemorrhages, which could indicate that the endothelial tissue had also undergone changes. OCT angiography results were within normal limits. In three patients, OCT revealed hyperreflexive lesions at the level of retinal ganglion cells and internal plexiform layers. These OCT results are similar to the results of examination of normal retinal vessels in terms of morphology, reflectivity, location and shadow, which lead the researchers to conclude that OCT results can often be misinterpreted, and the changes found during fundus ophthalmoscopy may signal of other systemic diseases. They stated the need for further research to confirm these results [21].

Experimental CoV retinopathy (ECOR) caused by neurotropic coronavirus strains

Neurotropic strains of coronavirus are of particular importance from the point of view of ophthalmology. There are two major strains studied: the JHM strain (JHMV) and the A59 strain (MHV-A59). They were originally isolated from paralyzed mice and have been found to cause extensive demyelinization and encephalomyelitis. The virus is capable of infecting glial cells, astrocytes, oligodendrocytes and microglia. Today, the retinal degeneration pattern caused by these strains is known as Experimental CoV Retinopathy (ECOR). In mice, presence of the virus in the retina and retinal pigment epithelium leads to infiltration of immune cells and release of pro-inflammatory mediators. The virus clearance is reached in the course of the first week of infection. However, autoantibodies to the retina and pigment epithelium cells form subsequently, with the result being progressing loss of photoreceptors and ganglion cells, as well as neuroretina thinning. According to these findings, retinal damage has an autoimmune component to it [14].

Effect of anti-coronavirus drugs on eye and vision

There have been suggested multiple SARS-CoV-2 treatment options. In addition to antiviral drugs, chloroquine (CQ), hydroxychloroquine (HCQ) and the like drugs are used widely. They are believed to reduce viral replication [22, 23]. Since therapeutic doses of these drugs are rather high compared to the maximum safe daily doses, taking them brings numerous toxic side effects, including those affecting the retina. According to the American Academy of Ophthalmology, the most significant toxicity-related risk factors the retina is exposed to in connection with these drugs are high doses and long duration of use [1, 2, 22, 23].

Researchers at the Royal College of Ophthalmologists in the UK tried to determine a safe dose and duration of CQ and HCQ therapy that would leave the retina unharmed. They recommend to not take more than 5 mg/kg/day of HCQ and keep the course shorter than 5 years. The researchers failed to determine a safe dose of CQ, but made a conclusion that those who received CQ for more than a year ran the risk retina damage [24].

It has been noted that in COVID-19 patients treated with high doses of hydroxychloroquine macular abnormalities have no visual symptoms [24-26].

The mechanism behind the toxic effect hydroxychloroquine has on the retina is unclear. Chloroquine and hydroxychloroquine were shown to strongly inhibit absorption capacity of the organic anion-transporting polypeptide 1A2 (OATP1A2), which is expressed by the human retinal pigment epithelium cells and participates in the complete recirculation of trans-retinol. The authors write about the possible effect of hydroxychloroquine on the visual cycle [25].

Both drugs are reported to damage the photoreceptor layer and the outer nuclear layer of the retina. Chloroquine can also damage inner nuclear layer of the retina. Light absorption and cone cell metabolism may also play a role in the damage. These mechanisms lead to such a characteristic maculopathy

as "bovine eye", which may develop after chronic exposure to both agents, even the safe doses thereof [22, 23]. It is important to note that both drugs are known for their binding affinity for melanin in the retinal pigment epithelium. This ability can contribute to the mechanism of manifestation of toxic effects [22].

Given the long half-life of these drugs, systemic clearance is delayed for several months after discontinuation. It is assumed that during this period the toxicity persists and may affect the severity of toxic maculopathy at the time of discontinuation. One study assessed visual acuity, SD-OCT and electroretinogram (ERG) data in patients that received HCQ. Six months after discontinuation, the patients had their visual acuity and ERG response improved, but no positive trends in the OCT-registered parameters. A further study was designed to examine 11 HCQ-induced retinopathy patients within 4 years after discontinuation. This work revealed that if a patient stops taking the drug before there is damage to the pigment epithelium, the retinopathy, as registered with SD-OCT, is limited to the first year only and does not affect the parafoveal region [26]. The researchers believe that preservation of the external limiting membrane is a favorable prognostic sign of hydroxychloroquine-induced retinopathy [26–27].

According to the analysis of the recommendations, doctors agree that when prescribing these drugs, all possible toxic effects should be taken into account and discussed with the patient. Those whose COVID-19 treatment plan included CQ or HCQ should visit ophthalmologists in case of any eye-related complaints [23]. The American Academy of Ophthalmology, the UK Royal College of Ophthalmologists and many other organizations recommend annual screenings for HCQ/CQ-induced retinopathy after 5 years of drug therapy. Patients who are exposed to risk factors should be screened before expiration of the said 5 years. Diagnostics should include computed perimetry, OCT and angiography. There is no screening duration figure mentioned in most recommendations, but it is likely the observation period should span several years, as the newly published statistical data show that toxic effects are manifested in 20–50% of people with more than 20 years of treatment [22, 27].

CONCLUSION

Coronaviruses can infect the eyes, causing a wide range of manifestations from anterior segment abnormalities such as conjunctivitis and anterior uveitis to vision-threatening conditions such as retinitis and optic neuritis. It is important to recognize that periodic mutations of the virus can dramatically change manifestations of the viral infection. Literature analysis shows that the data on SARS-CoV-2 transmission through the ocular tissue and eye damage are scarce, so there is a standing need for further research.

Despite the fact that the frequency of SARS-CoV-2 contraction through the surface of the eye is extremely low in the general population, it is important to remember that this is a route medical personnel and other categories may contract the infection. Accordingly, both ophthalmologists and patients should take precautions to minimize human-to-human contact transmission during the COVID-19 pandemic.

Further investigation of the mechanisms of action of the virus, as well as understanding of its connection to the symptoms in the visual domain, will help reinforce infection control measures, as well as allow understanding if eye tissue or even tear fluid may be used for diagnostic purposes. It is also important to identify new therapeutic approaches that minimize the use of toxic drugs in order to avoid the associated side effects on the eyes.

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KEY PARAMETERS OF AUTOLOGOUS BIOMEDICAL PRODUCT FOR CARTILAGE TISSUE REPAIR

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Repair of cartilage defects associated with injury or pathology is a clinically relevant problem. Chondral tissue, especially articular cartilages, has a poor regenerative potential. Inflammation triggers the growth of connective tissue, which cannot exert the normal function of the hyaline cartilage. This contributes to the progression of the pathology and eventually raises the need for surgery. At present, there are no pharmaceutical drugs capable of restoring the damaged cartilage. However, advances in cell-based technology hold promise for regenerative medicine. Reports describing fabrication of autologous cartilage transplants pose a special interest. A registration dossier of a biomedical cell product must contain the product's specifications, presenting the basic characteristics of the product that can be used to assess its quality. This review looks at a few basic parameters that can be used to verify the authenticity of the cell product derived from autologous chondrocytes and describe its specifications.

Keywords: chondrocytes, donor tissue, biomedicine, cell product, cell culture, biomarker expression

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КЛЮЧЕВЫЕ ХАРАКТЕРИСТИКИ АУТОЛОГИЧНОГО БИОМЕДИЦИНСКОГО ПРОДУКТА ДЛЯ КОРРЕКЦИИ ДЕФЕКТА ХРЯЩЕВОЙ ТКАНИ

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Восстановление дефектов хрящевой ткани после повреждений или при патологиях — одна из актуальных проблем в медицине. Физиологической и гистологической особенностями хрящевой ткани, особенно суставов, является пониженная способность к регенерации. Возникающее воспаление ведет к индукции образования соединительной ткани, которая уже не выполняет функции гиалинового хряща, что позволяет прогрессировать патологическому процессу и в итоге приводит к необходимости хирургического вмешательства. Фармацевтических препаратов, полностью востанавливающих поврежденную хрящевую ткань, на сегодняшний день на рынке нет. Между тем, большие надежды дает развитие клеточных технологий для нужд регенеративной медицины. В этой связи актуальны работы по созданию аутологичного хрящевого импланта для коррекции дефектов хрящевой ткани. При составлении регистрационного досье одним из основных документов является спецификация на биомедицинский клеточный продукт (БМКП). В ее основе лежит описание основных характеристик продукта, исходя из которых проводят контроль его качества. В настоящем обзоре представлен набор основных характеристик (показателей), которые можно использовать как для аутитентификации (процедуры проверки подлинности) разрабатываемого нами БМКП на основе аутологичных хондроцитов, так и для составления его спецификации.

Ключевые слова: хондроциты, донорский материал, биомедицина, клеточный продукт, культивирование клеток, экспрессия маркеров

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Hyaline cartilage repair poses a significant challenge due to the complex microarchitecture, avascular nature and poor regenerative potential of articular cartilage tissue, especially in advanced-age patients. Today, it is recognized that this problem requires a multifaceted approach involving the use of cell-based technology, new materials, gene engineering, growth factors, hormones, and drugs [1, 2].

Articular cartilage repair after an injury or in post-traumatic arthritis is one of the leading areas of orthopedic care. There are increasingly more patients with a history of maxillofacial or plastic surgery requiring articular cartilage repair. According to VADEMECUM, upwards of 21,000 rhinoplasties are performed in Russia every year. Articular cartilage injuries account for over 10% of all traumatic injuries; in most cases, the mandible is involved. One of the promising treatment options for injuries and defects of the ear and nose, including microtia, is chondrocyte implantation. Maxillofacial surgery is performed in different age groups, but, according to ISAPS (the International Society of Aesthetic Plastic Surgery), 65% of rhinoplasties are performed on female patients aged 19 to 34 years. Synthetic implants

used in reconstructive surgery cause delayed immunologic and inflammatory responses in 10–16% of cases [3].

Some companies have already started testing their products for reconstructive surgery. The University Hospital of Basel (Switzerland) has launched a clinical trial (ClinicalTrials.gov ID: NCT01242618) of an engineered cartilage graft for nasal alar reconstruction in patients with non-melanoma skin cancer. The graft was derived from the autologous human nasal chondrocytes cultured on a porcine collagen I/III membrane. So far, the first phase involving 5 patients has been completed.

Most developers use multicomponent systems for creating autologous cartilages suitable for implantation. For example, chondrocytes expanded in the medium containing the basic fibroblast growth factor FGF-2 and BMP-2 preserve their chondrogenic potential and form a high-quality, properly sized cartilage. A study reports that chondrocytes expanded in the presence of FGF-2 and cultured *in vitro* in a 3D biodegradable scaffold (polyglycolic acid, PGA) for 6 weeks formed a cartilage which contained 3.7 times more chondrocytes than a cartilage grown without FGF-2; the weight of the construct was 4.2 times greater and its glycosaminoglycan content was 2.8 times higher. The cartilage formed by the chondrocytes cultured in the presence of the bone morphogenetic protein BMP-2 and passaged in a medium supplemented with FGF-2 contained 1.5 more glycosaminoglycans and was characterized by their more homogenous distribution than a similar cartilage grown without BMP-2 [4]. In order to obtain a cartilage graft of an appropriate size and optimal mechanical properties, chondrocytes need to be cultured for 2 weeks prior to implantation.

In another study, expanded human nasal septum chondrocytes obtained from 4 donors were seeded on a Hyaff-11 scaffold, cultured *in vitro* for 2 or 4 weeks and then subcutaneously transplanted into immunodeficient mice. Two weeks after implantation, the elasticity of the cartilage precultured *in vitro* was 2.7 times higher than the elasticity of the construct transplanted immediately after cell seeding [5]. Fetal bovine serum (FBS) used to culture human nasal septum chondrocytes can be replaced with autologous human blood serum. Histopathology, immunohistology and biochemical evaluation of cell proliferation, glycosaminoglycan and collagen II content did not reveal significant differences between chondrocytes cultured in the presence of different serum types [6]. Importantly, the use of autologous serum reduces cell culture costs and the dangers associated with FBS, including the risks of immunogenicity or contamination with undetected agents, like prions that cause spongiform encephalopathy.

Among the advantages of autologous cartilage grafts (i.e., grafts harvested from the same patient) are good long-term cartilage survival, availability and immunotolerance. The downsides include the risk of donor site morbidity and graft resorption over time. The most common complication is warping typically seen in costal cartilage grafts [7]. Cartilage grafts take different shapes, from rectangular to trapezoidal to oval. Their size ranges from 1–2 mm to a few square cm. The graft is carved out of the harvested tissue samples and adjusted to a patient's parameters immediately during surgery. To achieve the desired curvature, small incisions can be made on the graft surface. It is possible to use articular cartilage grafts in 2 or more layers for improved strength. Grafts derived from the elastic ear cartilage can be folded to achieve better rigidity [8]. The optimal thickness of the graft is 1–1.5 mm. Reconstruction of the nasal dorsum is done with 1 mm3 pieces of the cartilage wrapped in Surgicel, fascia lata or temporal fascia and modelled in the recipient bed prepared in advance. Unwrapped grafts can proliferate, accumulate collagen after transplantation [9] and tend to resorb. Wrapped grafts are reported to exhibit signs of pronounced inflammation on a histopathologic examination [10].

A bioengineered cartilage graft has a few advantages over a conventional autologous graft: it requires less donor tissue and can be grown to a large size. This is especially important when there is a need for a repeat or revision surgery but the donor site has been depleted [8] and other donor sites are not available [11]. Using cell-based technology for engineering a graft identical to the native cartilage obviates the need for harvesting large volumes of human tissue, reduces the number of surgical interventions (for example, microtia repair normally takes 2 to 5 interventions), improves the cosmetic outcome, and makes the entire procedure less laborious. A bioengineered cartilage is expected to mimic its native counterpart in shape, size and mechanical properties.

Osteoarthrosis (OA) is the most common joint disorder affecting at least 20% of the world's population. Usually, the age of onset is above 40 years. Radiographic signs of OA are detected in 50% of people aged over 55 years and 80% of people aged over 75 years. OA of the knee joint (gonarthrosis) affects more women than men; by contrast, coxarthrosis (OA of the hip joint) is more prevalent in men than women [12]. According to the World Health Organization, OA of the knee and hip joints is the 11th leading cause of disability, and the number of patients with OA is continuously growing [13]. According to some estimates, OA of the knee joint accounts for 83% of all OA cases [14]. As the world's population is aging, the prevalence of OA is increasing.

At present there are no effective noninvasive and minimally traumatic pathogenetic treatments for gonarthrosis. Most treatments available are derived from hyaluronic acid (prosthetic synovial fluid). After a few years of symptomatic therapy, most patients end up needing a knee joint replacement. According to Global data, 33,000 knee replacement surgeries were performed in 2017 Russia. Depending on the prosthesis model, revision surgery (i.e. replacement of worn-out components of the prosthesis) is normally performed 5 to 10 years after the initial surgery. The service life of the prosthesis is approximately 15 years. OA of the hip joint often leads to femoral head osteonecrosis, necessitating total hip replacement, which is not recommended for young patients. Total hip arthroplasty is a costly and traumatic procedure; about 40% of the operated patients need a revision surgery within 10 years after the initial intervention. Immobilization leads to high mortality within a year in unoperated patients.

Today, cell-based medicinal products for articular cartilage regeneration are an alternative to classic reconstructive surgery involving subchondral drilling and abrasive arthroplasty. Some of such treatments are already available on the market, while others are currently undergoing clinical trials. In 2017, the EU witnessed the launch of Spherox (CO.DON AG), spheroids derived from autologous chondrocytes. Spherox is suitable for treating recent injuries of the knee joint of less than 10 cm^2 in size and has a few serious drawbacks: donor tissue is harvested arthroscopically, and another arthroscopy is needed for intraarticular implantation of the cultured spheroids. So far, the efficacy of Spherox for treating OA has not been demonstrated in comparative clinical studies. Other cell-based medicinal products available on the market are represented by allogenic and autologous derivatives of mesenchymal stem cells (MSC) isolated from adipose tissue or bone marrow, including Elixcyte by UnicoCell Biomed CO., Taiwan (culture-expanded allogenic adipose tissue MSC, phase 1–2 trial; Regenexx-SD by Regenerative Sciences, USA (non-cultured bone marrow

cells); ReJoin by Cellular Biomedicine Group, USA (adipose-derived mesenchymal stromal cells, phase 2 trial; RegStem by EMO Biomedicine Corporation, Taiwan (culture-expanded autologous mesenchymal stromal cells, phase 1 trial; JointStem by Nature Cell Co. Ltd., Korea (adipose-derived autologous mesenchymal stromal cells, phase 2 trial; StroMed by VivaTech International Inc., Netherlands (mechanically isolated stromal vascular fraction of adipose tissue, phase 2 trial). As a rule, these treatments are effective in very early stages of OA when its clinical manifestations are minimal or absent. This is due to the structural properties of the articular cartilage: cartilaginous tissue consists of the abundant extracellular matrix with few functional cells, i.e. chondrocytes that exhibit low plasticity and proliferative activity. This is why articular cartilages cannot heal spontaneously in physiological conditions.

Key requirements for donor tissue and cell isolation protocols

The source of a cartilage graft is the hyaline cartilage of the joint, the nasal septum, auricular or costal cartilage tissue. The transplant is expected to be easily removed should the need arise and not to irreversibly integrate into the surrounding tissue [3]. One of the main requirements for the graft is long-term size/shape stability. It is essential that the transplanted cartilage should not expand or change its shape over time, forming visible defects ("bosses"). The transplant must be resistant to fibrosis and resorption. This can be achieved if the transplant is composed of only chondrocytes with low proliferation potential and is devoid of chondroblasts. At the same time, the histological structure of the resultant cartilage must mirror the structure of a mature cartilage.

To maintain the compositional stability of the transplant, cartilage tissue should be harvested without the perichondrium. However, the border between the cartilage and the perichondrium is indistinct, and the harvested sample will inevitably contain a few chondroblasts [15]. The cartilage is composed of 2 layers that are visibly distinct under the microscope. The superficial layer contains elongated fibroblast-like cells oriented parallel to the surface. This layer is relatively abundant with collagen I. The deep layer is constituted by round cells.

Thus, to isolate chondroblast for further expansion, the harvested piece of cartilage is subjected to brief enzymatic incubation resulting in the digestion of its superficial layer; the detached cells are used for further expansion. With this approach, there is no need to mince the cartilage. To obtain chondrocytes for further culture, cells isolated during enzymatic incubation are removed, the cartilage is minced and enzymatic incubation is then continued for a few hours following the technique described in [16] or a similar technique. Cell yield increases with a patient's age [17], which might be explained by the lower density of the extracellular matrix in older patients. The proportion of viable isolated cells is the same in all age groups. Cell monolayer cultures can be maintained through 4 passages [18], the number of cells doubling with each passage.

Technologies for fabricating chondrocyte-based medicinal products

The articular cartilage contains few cells (5–10% of its volume) in comparison with the extracellular matrix. The area of a bioengineered cartilage must be comparable with the size of the tissue defect, as is the case with Co.don chondrospheres for the reconstruction of the knee joint cartilage. The thickness of the cartilage defect that can be repaired with the Co.don technology is similar to the thickness of the cartilage graft used in rhinoplasty (1–1.5 mm). This means there is a ready-for-use, well-established technique for cartilage size reduction and cultivation. Extrapolation of Co.don data shows that the approximately 40×10^6 chondrocytes are needed to create a graft for closing a 4 cm^2 cartilage defect (50 chondrospheres per 1 cm^2 of a knee joint defect; 200,000 cells per chondrosphere). Up to $1–1.5 \times 10^6$ cells can be obtained from 1 g of the harvested nasal septum cartilage [16]. In one of the studies, the nasal septum cartilage separated from the perichondrium was predigested with pronase or hyaluronidase and then finally digested with collagenase II. The optimal seeding density at which chondrocytes proliferate and remain viable for 10 days is 1×10^5 cells per culture flask.

On day 10, cells isolated from the superficial layer of the nasal septum cartilage and cultured in agarose start to lose their spindle shape, become more oval and can be assessed by staining with safranin O. The ratio between collagen II and I expression increases as the cells mature in a culture medium that does not contain any growth-stimulating factors. Some authors believe that chondrogenic factors TGFβ and BMP are not essential for the successful differentiation of cells into chondrocytes [15]. However, other researchers think that these factors increase the chondrogenic potential of chondrocyte cultures [19].

The presence of autologous serum can stimulate chondrocyte proliferation [20]. Supplementing the medium with 50 ng/ml CCN2/CTGF (CCN family 2/connective tissue growth factor) can increase by one and a half times proliferation of rabbit auricular chondrocytes and proteoglycan synthesis by these cells, as compared to the cells cultured in a medium containing only 10% serum [21]. The low oxygen environment of a bioreactor accelerates chondrocyte differentiation. The best differentiation is achieved at 5% DO (1% oxygen in the liquid phase) [22].

The majority of the applied technologies offer a 2-step procedure for cartilage engineering: chondrocyte expansion in a monolayer culture (the cells are reseeded 14 or sometimes 6–8 days after initial seeding) and creation of a 3D construct. In a monolayer culture, chondrocytes dedifferentiate when their proliferation is stimulated. It is not advisable to passage chondrocytes more than 4 times due to poor differentiation and predisposition to apoptosis [23].

In the second step, which takes about 7 days, a 3D tissue construct is grown on a biocompatible fibrous polymer scaffold (PGA etc.) or in a scaffold-free system using gelling polymers (alginate, ARC technology). Researchers working with 3D matrices think that chondroblasts attached to the fibers of a 3D matrix more readily arrange into a 3D structure of an articular cartilage and better differentiate into mature chondrocytes [23]. The ARC-technology facilitates maturation of fibroblast-like cells and promotes production of the extracellular matrix [24].

In another study, a porous HAp/ChS scaffold (collagen, hydroxyapatite and chondroitin sulfate) was used to engineer an ear cartilage [25]; such scaffold can assume the desired shape, and cells are distributed uniformly throughout its volume. In the cited study, the cells were cultured in a rotating bioreactor.

Importantly, as much as 75% if cells is lost during seeding into a scaffold. Given the proportions of cells and the extracellular matrix in a cartilage, the focus should be shifted from increasing the number of cells to stimulating extracellular matrix synthesis. So far, the biomechanical properties of bioengineered cartilage tissue are inferior to those of a native cartilage.

A group of researchers has proposed a technology for 3D chondrocyte culture that does not rely on biocompatible

polymers. Using layered chondrocyte sheets, the researchers were able to obtain a construct that had characteristics comparable to those of a native auricular cartilage [26–28].

This technology has been tested for safety and is now used for cartilage repair in Japan; so far, over 100 patients have received this treatment.

Sometimes the protocol for cartilage engineering includes one more step: maturation of the cartilage *in vivo* in immunodeficient (nude) mice. Despite the advantages, the method has serious limitations preventing it from mass use: it is difficult to guarantee that the end product will not contain any traces of murine tissue. However, it is still possible to monitor the maturation of a transplant in the recipient's body (for example, in cases when an auricular cartilage graft is grown for microtia repair).

The step of cell expansion in culture, which follows cell isolation, can be skipped: harvested cells can be immediately seeded into a scaffold. A histological examination of bioengineered tissue grown in a PGA scaffold was conducted after 28 days of cell culture [29]. Using immunohistochemical analysis, collagen levels, DNA content, and sulfated glycosaminoglycans (sGAG, assessed by staining with toluidine blue) were measured. At passage 0 (the seeding of cells into a PGA scaffold right after harvesting), the growing cartilage was comparable with the cartilage tissue derived from precultured cells and had higher DNA and sGAG content. However, the amount of cells isolated from a human nasal septa and used for immediate seeding into a scaffold was insufficient to grow a properly sized cartilage.

Chondrocytes can arrange into a cartilage-like structure in the absence of a scaffold. However, they grow slowly. A study reports that cells seeded at the density of 1.6×10^6 chondrocytes per 1 cm^2 and grown for 10 weeks generated a 291-μm-thick construct; of that size, calcified tissue amounted to 77 μm [30].

Basic markers for quality control

Cells cultured as a monolayer are elongated and have a chondroblast-like phenotype. If cells are cultured at high density in 3D scaffolds, they acquire a round shape and start to resemble chondrocytes. On day 7 of high-density culture in a 3D matrix, the cells become round, with large euchromatic nuclei, a few nucleoli and a well-structured cytoplasm. As early as day 1 of culture in a 3D fibrous matrix, numerous cell contacts can be detected. On the periphery, the cells look more spherical, but in the center they are flatter. On day 7, cartilage nodules appear [23]. The immunohistochemical analysis showed that cell cultures derived from the nasal septum cartilage express collagen I and CD44, whereas expression of collagen I and aggrecan is significantly lower [16].

Chondrocytes isolated from human cartilaginous tissue express CD105, CD44 and CD73 and are negative for CD146. The receptor for hyaluronic acid CD44 is abundantly expressed in young tissue; as the cartilage becomes thicker CD44 expressions declines. Cartilaginous tissue was shown to express Sox-9 RNA (the gene is considered to be the marker of chondrogenesis). It also lacked expression of SBFA-1 RNA, the marker of ossification [15].

It is recommended to measure glycosaminoglycan (GAG) content instead of collagen levels in a growing 3D construct to evaluate the amount of the extracellular matrix because GAGs are more abundant and their synthesis starts earlier than collagen synthesis. After 2 weeks of culture, GAG levels were 7 μg per 40,000 seeded cells [31].

Chondrocyte differentiation can be assessed using commercial monoclonal antibodies [32]. Chondrocyte maturation can be assessed with surface markers expressed by mature and immature cells during culture. The expression of CD44 and integrin alpha-5 is considered the most specific for immature chondrocytes and chondroblasts [33]. It is also useful to measure collagen I and II, S100, aggrecan, sox 6, sox 9 [34], cartilage-expressed gene 1, or CRTAC 1 [35], and Ca^{2+} release-activated Ca^{2+} channel [36].

Key characteristics of bioengineered tissue

The biochemical composition of a cartilage in a 45 to 47-year old human per 1 g cartilaginous tissue is as follows: 83–88 mg of collagen and 27–29 mg of sulfated glycosaminoglycans; the total amount of cells is 25–26 million [29].

Histologically, a healthy cartilage is composed of chondrocytes differing in shape and metabolic activity. The cells are more round in the center of the cartilage, as compared to its periphery. The cell to the extracellular matrix ratio declines from the periphery to the center. The cartilage lacks collagen 1, but contains collagen 3 [37].

PoC studies of bioengineered tracheal cartilages derived from a nasal ovine cartilage analyzed the histological appearance of the resultant 3D products stained with hematoxylin-eosin and safranin O. Cartilaginous nodules were detected in the bioengineered tissue surrounded by the extracellular matrix. The primary biomechanical property of the cartilage is compressive stiffness; in the study, it ranged from 0.44 and 0.7 MPa depending on orientation. During storage, this parameter increases by approximately 50% a month [38, 39]. In another study, the best compressive stiffness demonstrated by the samples was 0.0056 MPa, which is way inferior to the parameters of native cartilage tissue [40]. If the protocol for cartilage growth is adjusted to include cartilage maturation in nu/nu mice, the resultant construct assessed after 30 days of culture has improved stiffness.

To study how well the bioengineered cartilage can recover its shape, 10 × 2 × 1 mm strips of a construct obtained with the ARC-technology (10 weeks of culture in a scaffold) were loaded into the controlled environment of a bending bioreactor where stress was applied to the samples using a 5 mm loading post. The angle between the margins of the strip was measured immediately after applying stress (0h), 2 h and 24 h after unloading. In other words, shape retention was assessed (0% — complete recovery of shape, the opening angle is 180°). Differences in this parameter between the native cartilage and the bioengineered construct were insignificant [41]. Dynamic flexural stiffness (resistance to bending) of the construct was 0.014 ± 0.019 N/mm vs 0.19 ± 0.15 N/mm of the native tissue [42]; hydroxyproline content was similar between the bioengineered and native tissues.

The average GAG content in the construct produced with the ARC technology was 0.318 ng per cell. The average collagen II content was 0.2 μg/mg wet tissue weight; collagen I content was very low. GAG content per 1 mg wet tissue weight was 10.74 μg/mg before implantation into immunodeficient mice, 8.86 μg/mg after 30 days of *in vivo* culture and 2.73 μg/mg after 60 days of *in vivo* culture. Collagen II content was 0.02 μg/mg wet weight before implantation, 0.78 μg/mg after 30 days of *in vivo* culture and 1.44 μg/mg after 60 days of *in vivo* culture. Collagen I content was below the detection threshold, similarly to the native cartilage. The proportion of viable cells in the construct was above 90%. Still, the mechanical properties of the construct were inferior to those of the native cartilage [40].

CONCLUSION

There is a need for new protocols that can improve the yield of cells suitable for culture from donor tissue. Culture protocols are expected to produce a construct that mimics native tissue in its morphology, molecular (expression of glycosaminoglycans, collagen II, aggrecan), physiological and mechanical properties. Although costly technologies are required to reduce the probability of cross-contamination when working with autologous tissue and such work poorly scalable, it is still possible to create a product with reduced immunogenicity, posing little risk for infection. Developing a technology for producing implants mimicking a hyaline cartilage that can rapidly restore the function of the cartilage, allow the patient to return to the usual level of physical activity and minimize treatment costs is a pressing concern [43].

It is advisable to use 3D culture technologies for creating a neocartilage construct either by building chondrocyte layers consecutively or by shaping 2D cell cultures into spheroids with subsequent maturation *in vitro* or by using a combination of these 2 methods. In this case, chondrocytes retain their mature differentiated state and produce the extracellular matrix. With such products, there is no need to use additional scaffolding, which requires more clinical trials, complicates the technology and increases costs.

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SARS-COV-2 IN THE CONTEXT OF CORONAVIRUSES AND ANIMAL MODELS OF COVID-19

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Some human coronaviruses that share genetic similarity are known to infect other mammals. A host can harbor several coronaviruses, which creates favorable conditions for recombination and eventually results in the emergence of new viral strains and species. This review looks at SARS-CoV-2 in the context of other coronaviruses and their evolution, with a special focus on possible host jumps and adaptation of the virus to its new hosts. To understand these phenomena, it is essential to know the ecological relationships between the host and other organisms. Candidate COVID-19 models are not limited to the organisms and laboratory animals previously used to study SARS and MERS. The diversity of SARS-CoV-2 hosts suggests there is a wide range of candidate animal models for studying COVID-19 that might be suitable for testing drugs and vaccines against this infection. Considering the diversity of coronaviruses, integrated medical, veterinarian and zoological studies might help to speed up the development of tools for combating coronaviral infections and prevent future epidemics.

Keywords: coronavirus, SARS-CoV-2, COVID-19, animal models, viral infections, transmission, epidemic, zoonotic diseases, reservoir

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SARS-COV-2 В КОНТЕКСТЕ КОРОНАВИРУСОВ И ЖИВОТНЫЕ МОДЕЛИ ДЛЯ ИЗУЧЕНИЯ COVID-19

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Среди коронавирусов, инфицирующих человека, известен ряд генетически близких видов, поражающих других млекопитающих. В одном организме могут сосуществовать несколько коронавирусов, что создает условия для рекомбинации, приводящей к появлению новых вирусных штаммов и видов. В данном обзоре представлены особенности SARS-CoV-2 в контексте других коронавирусов и их эволюции. Особое внимание уделено возможности перехода коронавируса на новых хозяев и его адаптации, для чего важно понимать экологические связи хозяев с другими живыми существами. Модельными объектами для изучения COVID-19 могут быть не только испытанные на SARS и MERS организмы и популярные лабораторные животные. Разнообразие поражаемых SARS-CoV-2 животных свидетельствует о наличии широкого спектра потенциальных модельных объектов для изучения COVID-19, способных оказаться эффективными при разработке лекарств и вакцин. С учетом разнообразия коронавирусов взаимная интеграция медицинских, ветеринарных и медико-зоологических исследований может ускорить разработку средств борьбы с коронавирусными инфекциями, а также способствовать предупреждению новых эпидемий.

Ключевые слова: коронавирус, SARS-CoV-2, COVID-19, модельные животные, вирусная инфекция, передача вирусов, эпидемия, зооноз, естественный резервуар

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SARS-CoV-2 is one of the 3 coronaviruses to have caused an epidemic among humans in the 21st century. Notably, all of those 3 viruses were zoonotic [1]. This underscores the dangers of zoonotic infections to humankind. Coronaviruses have a capacity for recombination and therefore can infect different species. This review looks at SARS-CoV-2 in the context of other coronaviruses that pose a threat to mammals in general and humans in particular. Similar to the studies of SARS-CoV and MERS-CoV, veterinary research of animal coronaviruses, including FCoV (feline infectious peritonitis), CCoV (canine viral enteritis), SADS-CoV (swine acute diarrhea syndrome) and some others, can yield invaluable data for countering SARS-CoV-2.

Developing effective and convenient experimental models of COVID-19 is a pressing concern because animal models are indispensable for studying the pathogenesis of the disease and testing candidate drugs and vaccines.

General characteristics of SARS-COV-2

An RNA virus that caused the pandemic of 2020 and was termed 2019-nCoV or SARS-CoV-2 is a member of the Coronaviridae family. Today, 7 Coronaviridae viruses are known to infect humans; of them 3 are associated with acute respiratory syndromes (SARS-CoV, MERS-CoV and SARS-CoV-2) and 4 (HCoVs) cause only mild respiratory symptoms (Table 1). According to international reports, all the 4 HCoVs circulate in the human population all year round and are characterized by seasonal incidence peaks [2, 3].

SARS-CoV-2 causes an often asymptomatic disease called COVID-19 [4]. The signs and symptoms observed in patients with mild or moderate COVID-19 remind those of acute respiratory infections and seasonal flu, hampering the diagnosis. Severe COVID-19 can lead to complications, including acute respiratory distress syndrome and multisystem disorders.

The primary route of SARS-CoV-2 transmission is through droplets produced by an infected individual during coughing, sneezing, talking or breathing. This mode of transmission is typically seen in humans and between humans and domestic animals. The novel coronavirus can also spread through fomites, which are objects and surfaces contaminated with biological fluids of infected patients containing viable SARS-CoV-2

Table 1. Diversity of human coronaviruses

Virus	Genus	Natural reservoir	Intermediate host	Transmission route	Receptor
HCoV-229E	*Alphacoronavirus*	Bats	Camels	Droplets/aerosols, fomites	APN
HCoV-NL63			Unknown	Droplets/aerosols, direct contact	ACE2
HCoV-OC43	*Betacoronavirus*	Rodents	Cattle		9-O-acetyl-N-acetylneuraminic acid
HCoV-HKU1			Unknown		
MERS-CoV		Bats	Camels		DPP4
SARS-CoV			Palm civets (*Paradoxurus hermaphroditus*)	Droplets/aerosols, direct contact, fecal-oral route	ACE2

virions [5]. It is reported that the pathogen has been detected in wastewater, so it is possible that exposure to contaminated wastewater may result in SARS-CoV-2 infection [6]. Additionally, SARS-CoV-2 RNA has been detected in blood, mucus, saliva, urine, feces [5] and sperm [7. The definitive factor ensuring the spread of the virus through direct contact is its viability outside the host. According to the literature, the reported viability of the novel coronavirus varies from a few hours to a few weeks, depending on the type of contaminated surface and some environmental factors.

The mechanism used by SARS-CoV-2 to invade the host cell is still debatable. There are a few possible entry points, including 2 cell receptors CD147 [8] and GRP78 [9]; however, the dominant cell entry mechanism is through the membrane receptor ACE2 [10]. The S-protein, which forms spikes on the surface of the viral nucleocapsid, anchors to ACE2, and the subsequent cell entry is mediated by the transmembrane serine protease 2 (TMPRSS2) [11]. A similar mechanism is employed by SARS-CoV [12].

ACE2 is expressed in more than 150 different cell types found in almost all human tissues and organs [13], but its expression levels vary depending on the cell type. ACE2 is present on the membranes of type II pneumocytes, small intestine enterocytes, endothelial cells of arteries and veins, and smooth muscle cells of most human organs. Given that SARS-CoV-2 uses ACE2 to enter the cell, one can expect that some signs of COVID-19 will be observed in ACE2-expressing tissue.

Evolution of SARS-COV-2

It is reported that HCoVs are descended from animal coronaviruses. For example, SARS-CoV, MERS-CoV, HCoV-NL63 and HCoV-229E are related to bat coronaviruses, whereas HCoV-OC43 and HKU1, to rodent coronaviruses [1]. SARS-CoV-2 is likely the product of genetic recombination that occurred in a natural reservoir, the Chinese population of bats [14]. The SARS-CoV-2 genome shares 89% sequence homology with SARS-like-CoVZXC21 and 96% sequence homology with RaTG13.

Being an RNA virus, SARS-CoV-2 has 2 evolutionary strategies: 1) genetic drift or natural selection of mutations and 2) exchange of genetic material with other viruses through recombination [15].

Between December 2019 and September 2020, over 18,500 SARS-CoV-2 genomes were sequenced. Based on the sequencing data, it was concluded that the novel coronavirus is relatively conserved. This means that future vaccines against SARS-CoV-2 might be equally effective against any of its variants [16]. Most mutations detected in SARS-CoV-2 genome do not affect the properties of the pathogen or reduce its pathogenicity/virulence toward humans. The rate of such mutations, including D614G, can be explained by the founder effect.

Structurally, SARS-CoV-2 is a spherical or pleomorphic enveloped particle containing single-stranded positive-sense RNA. SARS-CoV-2 RNA is complexed with the nucleoprotein inside the viral capsid formed by the matrix protein [17]. The club-shaped spikes of the glycoprotein known as the S protein protrude from the viral envelope. The S protein binds to the membrane of the host cell and mediates the invasion.

Numerous animal studies have demonstrated that coronaviruses frequently undergo genetic recombination. For example, S-protein recombination seems to be a common event in feline and canine coronaviruses [18]. The S-protein is a membrane glycoprotein composed of 2 subunits: S1 and S2. The S1 subunit enables the virion to latch onto the host cell exploiting the interactions between its receptor-binding domain (RBD) and the receptor located on the surface of the host cell. The RBD-encoding gene region is the most variable part of the coronavirus genome. The genetic flexibility of the S protein and especially its RBD might allow the pathogen to less specifically bind to ACE2 receptors in a variety of animal species and thus expand the range of possible hosts [14]. Mutations in the S protein might induce conformational changes, which, in turn, affects viral antigenicity. So far, a few mutations have been discovered in the S1 receptor binding region but they have not undermined the ability of the virus to bind to ACE2 in humans, pigs, civets, and bats [19]. Recombination between gene regions coding for the S1 and S2 subunits of the S protein was deemed as one of the major mechanisms facilitating the emergence of human SARS-CoV strains from bat and civet ancestors [20].

Because SARS-CoV-2 is transmitted more rapidly that it evolves, its population is becoming more homogenous, with a median of 7 nucleotide substitutions between genomes. There is evidence of purifying selection, but little data is available to suggest diversifying selection; the rates of nucleotide substitutions are comparable between structural and non-structural genes [16]. Most mutations acquired by the virus are phenotypic and thus provide information on the geographic and population origin of the viral lineage.

The S protein of SARS-CoV-2 effectively binds to ACE2 receptors in humans, ferrets, cats and other mammals sharing high receptor homology [21]. The remarkable diversity of species susceptible to SARS-CoV-2 suggests that the pathogen can cross the species barrier and encounter other coronaviruses, which might result in a recombination event and thus give birth to novel viral strains and species. In the past 20 years, 3 coronaviruses have spilled over from zoonotic reservoirs; this underscores the need for surveillance of animal coronaviruses [22], the importance of studying mutations that allow zoonotic viruses to perform a host jump and the usefulness of medical zoology research.

Recombination events among HCoVs have been amply described in the literature [23]. For example, the screening of specimens obtained from Kenyan bats allowed researchers to identify a few viruses exhibiting genetic similarity to HCoV-NL63 and HCoV-229. These viruses were reported to have an eventful history of genetic recombination, including 2 interspecies recombination events involving the S-protein gene. This suggests that the S-protein gene might be a recombination hot spot in coronavirus genomes [24].

Animal coronaviruses are a potential threat to humanity

Many mammalian coronaviruses have been well studied and characterized by veterinarian scientists. For example, it is known that β-coronaviruses encompass human viruses HCoV-OC43 and HCoV-HKU1 that cause acute respiratory infections in humans and a number of other viruses that infect dogs, cats, cattle, pigs, horses and camels. HCoV-OC43 and bovine BCoV share 95% sequence homology, whereas SARS-CoV-2 shares almost 96% sequence homology with RaTG13 (member of the SARSr-CoV group) isolated from the horseshoe bat (Rhinolophus affinis). Viruses genetically close to SARS-CoV-2 have been isolated from other bats and palm civets (Nandinia binotata) [15]. However, although SARS-CoV-2 and bat CoV RaTG13 share almost 98% homology in the sequences coding for the S protein, the SARS-CoV-2 genome contains an insertion of a furin cleavage site (RRAR) in the S1/S2 region. This multibasic cleavage site might be associated with the high virulence of the novel coronavirus [19]. A virus related to SARS-CoV-2 has been isolated from pangolins (Manis javanica), which is why these animals were thought to be an intermediate host for SARS-CoV-2 [25]. Animal hosts of β-coronaviruses are potential models of infectious disease caused by this group of viruses, including SARS-CoV-2. Notably, over time intermediate hosts can become natural reservoirs for coronaviruses, whereas viruses predominantly harbored by intermediate hosts can accumulate mutations independently. Besides, in the intermediate host the virus can accumulate mutations allowing it to successfully invade the final host. If the natural host is infected by different populations of the same viral species, recombination between these populations will drive the emergence of new strains [20].

Bats harbor a greater diversity of zoonotic viruses than other mammals [26]. The list of viruses hosted by bats includes relatives of SARS-CoV, MERS-CoV, HCoV-229E, HCoV-NL63 [27], and SARS-CoV-2 [25]. The fact that bats are lowly susceptible to infectious pathology caused by the viruses they host requires thorough investigation. But reports of coronaviruses crossing the species barrier [1] raise the need for close wildlife disease surveillance and research into the potential routes of viral transmission between species, because each host jump increases the odds of a fundamentally new recombination event associated with the virome of the host.

A host can be simultaneously infected with several coronaviruses, which creates favorable conditions for recombination and affects the clinical picture. Coinfections aggravate the course of a primary disease. In human hosts, SARS-CoV-2 can cooccur with other viruses, including coronaviruses that cause respiratory infections [28]. The most common SARS-CoV-2 coinfection is influenza A virus. Respiratory coinfections are negatively correlated with the accuracy of COVID-19 diagnosis, and clinical manifestations of COVID-19 do not always raise suspicions about the presence of another respiratory (viral, bacterial or fungal) pathogen, which may result in the wrong treatment choice. Unfortunately, coinfections in patients with COVID-19 remain heavily understudied [29]. Coinfection can contribute to the mutability of the coronavirus. Coronaviruses coexisting in one host undergo frequent recombination events and mutate actively [24]. So far, of 39 currently known coronaviruses [30] 7 are capable of infecting humans. These viruses pose a threat to agriculture and human health. Identifying the reservoirs of zoonotic pathogens is crucial to effective disease control and prevention [25].

Animal models of COVID-19

Animal models are indispensable for conducting preclinical trials of candidate drugs and vaccines and studying the pathogenesis of SARS-CoV-2 infection. Since the clinical manifestations of COVID-19 differ significantly among the infected individuals, it is important to create models reflecting different degrees of disease severity. This will allow researchers to preclinically assess the efficacy of candidate drugs depending on the severity of the disease. Studying the diversity of species that host the virus in question might help to find a suitable animal model. Animals in which the virus replicates but does not cause overt pathology are reservoirs for the infection; their surveillance is critical for preventing the outbreaks of the infection. Animals that can transfer the virus on their skin or fur constitute a separate category. For example, SARS-CoV-2 RNA has been detected in the biological samples of domestic dogs and cats, tigers and lions [15]. However, a positive PCR test does not prove that the tested animal is sick or is the carrier of viable virions. Nevertheless, the fact that the virus can be transmitted from humans to domestic animals is a worrying sign [15], although there were no reports of animal to human transmission.

Initially, the search for animal models of COVID-19 focused on the animals that had been previously regarded as candidate models for SARS and MERS. Unfortunately, none of them were fairly suitable to study these two viruses [31].

Attempts were made to study SARS-CoV replication in Syrian and Chinese hamsters, civets and non-human primates (NHP), such as rhesus monkeys, crab-eating macaques, African green monkeys, etc. [32]. Mice and ferrets were more susceptible to SARS-CoV infection but resistant to MERS-CoV, due to the properties of their DPP4 receptors [25]. Rabbits were not investigated as a potential model of SARS-CoV [31] and turned out to be an unsuitable research model for MERS [33]. A study demonstrated that ferrets (*Mustela furo*) and domestic cats (*Felis domesticus*) were susceptible to SARS-CoV and could effectively spread the virus to other noninfected animals they were housed with [34]. Likewise, domestic cats and ferrets can be infected with and spread SARS-CoV-2 [35], which makes them a promising SARS-CoV-2 candidate model.

American mink (*Neovison vison*) bred on fur farms are susceptible to SARS-CoV-2, which they presumably contracted from humans [36]. Thus, mink can be a good animal model for studying COVID-19 and other coronaviruses capable of binding to ACE2. Advantageously, there are well-established housing and care protocols for mink and ferrets. Mink can be used to model severe and moderate COVID-19. However, there are still a few issues related to the housing of these animals in a laboratory environment [37].

Tigers and lions have been reported to develop COVID-19 symptoms [15]. The fact that two distant families of the mammalian order *Carnivora*, *Mustelidae* and *Felidae*, can so easily contract the virus and develop COVID-19 indicates that the wide variety of animal species can act as a reservoir

Table 2. Model animals

	Models		SARS-CoV-2					
Animal species	SARS	MERS	Symptoms	Overt pathology	Antibodies	Advantages	Disadvantages	References
Syrian hamster (*Mesocricetus auratus*)	Yes	Does not replicate	Yes	Yes	Yes	The virus can be passed on from one animal to another. It replicates in and causes serious damage to the lungs, brain, olfactory bulb. Syrian hamsters produce antibodies against SARS-CoV-2 that neutralize the virus in other infected Syrian hamsters following convalescent serum transfusion. The virus is detected in the liver, kidneys, spleen, heart, intestines, salivary glands, lymph nodes	Rapid clearance of the virus. Pathology was less pronounced in naturally infected hamsters than in the animals with experimentally induced infection	[25, 33, 38–40]
Transgenic mice with human hACE2 receptor	Yes	Mice with human hDPP4 receptor	Yes	Yes	Yes	The virus replicates in the lungs, causing pneumonia. Inflammation is moderate	The virus does not affect other organs; coagulopathy does not develop. High costs	[25, 31, 33, 41]
Wild type mice without human receptors	Yes	No DPP4	Yes	No	No	No advantages	Wild type mice are not susceptible to the virus. Its replication is negligible	[25, 31, 33, 41]
Domestic cat (*Felis cattus*)	Yes	N/A	Yes	Yes	Yes	The virus is transmitted among cats. The virus replicates in the respiratory tract, tonsils and intestines. Kittens suffer more pronounced organ damage	Adult cats are significantly more susceptible to the virus than kittens	[33, 35]
Domestic ferret (*Mustela putorius furo*)	Yes	Does not replicate	In some ferrets	Yes	Yes	Transmission is possible	The virus replicates in the upper respiratory tract and intestines, but not in the lungs	[25, 31, 33, 35, 38]
Domestic dog (*Canis lupus familiaris*)	N/A	N/A	No	No	Not in all cases	No advantages	Low susceptibility to the virus; dogs with experimentally induced infection do not transmit the virus to other dogs	[35]
Domestic pig (*Sus scrofa domesticus*)	Failed	Failed	No	No	Conflicting data	No advantages	Not susceptible to the virus	[33, 35, 37]
Crab-eating macaque (*Macaca fascicularis*)	Yes	Yes	Yes	Yes	Yes	The virus replicates in the lungs and causes pneumonia	High costs, low availability, low levels of viral RNA	[31, 33, 38, 42]
Rhesus monkey (*Macaca mulatta*)	Yes	Yes	Yes	Yes	Yes	Increased cytokine expression. The virus replicates in the lungs and causes pneumonia. Viral RNA is detected in the early stages of the disease in the lungs, trachea, bronchi, spleen, stomach, rectum, bladder and uterus	High costs, low availability	[31, 38, 42]
Green monkey (*Chlorocebus sabaeus*)	Yes	Yes	Yes	Yes	N/A	A well-established model for many infectious pathologies. Model animals develop pneumonia	High costs, complexity, low availability. Clinical manifestations are very mild	[31, 33, 38, 42]
Common marmoset (*Callithrix jacchus*)	Yes	Yes	In some marmosets	No	No	The virus is detected in the blood	High costs, low availability. The virus is not detected in the lungs and does not cause pneumonia or severe lung pathology	[31, 33, 38, 42]

Note: N/A – data not available

for SARS-CoV-2 [34]. It is possible that some of them might become a new effective model for COVID-19. More different mammals need to be investigated in order to identify new potential sources of the infection and find suitable research models. Table 2 describes a few animal models for SARS-CoV-2.

Northern treeshrews (*Tupaia belangeri chinensis*) and Egyptian fruit bats (*Rousettus aegyptiacus*) were also investigated as candidate models of SARS-CoV-2 but they did not develop any pathology following a challenge with the coronavirus, although the virus was detected in the multiple organs of these animals [25]. Therefore, the northern treeshrew and the Egyptian fruit bat do not hold promise as COVID-19 models.

The susceptibility of nontransgenic mice to the coronavirus can be significantly affected by their genetic traits unrelated to ACE2 [25], which may skew the clinical picture in a way that cannot be predicted.

Alpacas (*Vicugna pacos*) and dromedary camels (*Camelus dromedarius*) were used as the first MERS models [33, 43]. But because the upkeep of dromedary camels is quite challenging and these animals can pass the infection to humans, researchers had to give up the idea of using them as a MERS model. Alpacas infected with MERS-CoV remained clinically healthy although they did produce antibodies [5]. Since there were more convenient animal models, the use of tylopods for studying SARS-CoV-2 was eventually discontinued.

Syrian hamsters turned out to be the most effective and cheap model of COVID-19. Cats and ferrets might hold some promise but their upkeep is more difficult. Despite the absence of data, mink are considered to be a promising model for SARS and MERS. NHP models are vigorously used in preclinical trials of candidate drugs and vaccines against COVID-19.

CONCLUSION

The diversity of coronaviruses poses a serious threat to epidemiologic safety. Future pandemics can be prevented using an integrated approach to medical, veterinarian and zoological studies. In the 20th century, the effective surveillance of zoonotic infections contained the spread of zoonotic viruses from wildlife to humans. Knowing the routes of viral transmission is as important as understanding the coevolution of the virus and its host. Studies of animal coronaviruses might provide invaluable data that will serve as a starting point in researching SARS-CoV-2 and other human coronaviruses. Animal models are useful in modeling human diseases, studying the progression of the disease and exploring the properties of the virus. Expanding the range of model animals will allow us to find the optimal models for studying the pathogenesis of COVID-19 and testing candidate drugs and broaden our research potential needed to counter new infections in the future.

Using a systemic biological approach to the analysis of viral diversity and the reconstruction of the interactions between the virus and its host under all possible outcomes, we will be able to effectively contain potential threats and prepare for new pandemics. The human body is an ecosystem, so studies looking into the interactions between the virus and its human host should take into account the interactions between the virus and the human microbiota, as well as the probability of recombination with viruses constituting the human virome. Humans are part of terrestrial ecosystems, so it is important to trace the transmission of the virus from animals to humans and from humans to animals as new mutant viral strains can be passed back from its new host to the human population.

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ROLE OF HEREDITY, ENDOGENOUS AND EXOGENOUS FACTORS IN GASTRIC CANCER

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Gastric cancer (GC) usually has an unfavorable prognosis: the five-year survival rate is 20–30% in most world regions. Timely diagnosis and prevention of risk factors may reduce mortality from GC. This review discusses the meta-analyses of 40 endogenous and exogenous factors associated with GC. GC is significantly associated with family history; dietary preferences (increased consumption of roast and smoked red meat, hot foods, pickles, salt (over 5–6 g/day), nitrates (over 20 mg/L drinking water); lifestyle (smoking, opium use, strong alcohol, beer, stress); some diseases including gastroesophageal reflux disease, diabetes mellitus, obesity, and autoimmune disorders; infections (*Helicobacter pylori*, human papillomavirus, Epstein-Barr virus); ionizing radiation, and professional hazards. Data suggesting associations between the risk of GC and the consumption of coffee, tea, high-fat foods, simple carbohydrates, folic acid, sleep duration, and blood cholesterol turned out to be conflicting due to the inconsistencies of the results between cohort and case-control studies. About 3% of all gastric cancers are linked to hereditary syndromes associated with pathogenic variants of *CDH1*, *STK11*, *SMAD4*, *BMPR1A*, *TP53*, *MYH*, *APC*, *PTEN*, *ATM*, *BRCA1*, and some other genes.

Keywords: gastric cancer, risk factors, polymorphism, hereditary syndrome, occupational hazards

Author contribution: Ershov PV performed literature search and wrote the draft of the manuscript; Veselovsky EM performed literature search, wrote the *Genetic factors for GC risk* section and edited the manuscript; Konstantinova YuS performed literature search, proposed the concept of the study and edited the manuscript.

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ВКЛАД НАСЛЕДСТВЕННОСТИ И СОВОКУПНОСТИ ЭНДОГЕННЫХ И ЭКЗОГЕННЫХ ФАКТОРОВ РИСКА В РАЗВИТИЕ РАКА ЖЕЛУДКА

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Прогноз рака желудка (РЖ) обычно неблагоприятен: пятилетняя выживаемость в большинстве регионов составляет 20–30%. Выявление злокачественного новообразования на ранних стадиях, так же как и своевременное исключение факторов риска, помогут снизить смертность от РЖ. В обзоре обсуждаются данные публикаций по мета-анализу 40 эндогенных и экзогенных факторов, связанных с РЖ. Статистически значимый риск РЖ был ассоциирован с семейным анамнезом; некоторыми диетическими особенностями (высокое потребление жареного и копченого красного мяса, горячей пищи, маринованных продуктов, поваренной соли (свыше 5–6 г/сут.), нитратов (свыше 20 мг/л питьевой воды); стилем жизни (табакокурение, потребление опиума, крепкого алкоголя и пива, стресс); такими заболеваниями, как гастроэзофагеальная рефлюксная болезнь, сахарный диабет, ожирение, аутоиммунные нарушения; инфекциями (*Helicobacter pylori*, вирус папиломы человека, вирус Эпштейна–Барр); ионизирующим излучением; профессиональными вредностями. Данные о связи риска РЖ с потреблением кофе, чая, пищи с высоким содержанием жиров и быстроусваиваемых углеводов, фолиевой кислоты, продолжительностью сна, содержанием холестерина крови оказались противоречивыми, вследствие отсутствия согласованности результатов когортных исследований и «случай–контроль». Около 3% всех случаев РЖ обусловлены наследственными синдромами, ассоциированными с патогенными вариантами генов *CDH1*, *STK11*, *SMAD4*, *BMPR1A*, *TP53*, *MYH*, *APC*, *PTEN*, *ATM*, *BRCA1* и др.

Ключевые слова: рак желудка, фактор риска, генетический полиморфизм, наследственный синдром, профессиональная вредность

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Gastric cancer (GC) is usually diagnosed in advanced stages. The neoplastic transformation of gastric mucosa has a complex nature shaped by the interplay of endogenous and exogenous factors, from genetic polymorphisms to lifestyle choices and occupational hazards. Early detection and elimination of modifiable high-risk factors reinforced by the promotion of behaviors that can lower the risk of GC is the mainstay of cancer prevention strategies. The leading high-risk factors for GC are male sex (men are twice as likely to develop GC than women), *Helicobacter pylori* (*H. pylori*) infection, family history of cancer and smoking.

Across continents, the highest incidence of GC is observed in East Asia, followed by Central and Eastern Europe, South America, Southern Europe, Northern Europe, Central Asia, North America, and Africa [1]. Within countries, differences in GC incidence are linked to the ethnic composition of the resident population, culture, climate, and regional geochemistry. Survival depends on the stage of the disease at diagnosis, its classification category and molecular subtype. GC usually has a poor prognosis: the 5-year survival rate varies from 20% to 30% in most world regions [2], except Japan, where it exceeds 70% for stages I and II [3]. The high survival rate observed in Japan may indicate the success of mass screening programs for early cancer detection *in situ* that can prevent invasive cancer.

Today, public health systems all over the world are making progress in treating *H. pylori*, one of the key factors predisposing to GC, and raising health awareness among the population. Owing to health education, the mortality from GC declines by 3% annually in many countries, including Russia [1].

This review discusses over 100 meta-analyses of cohort and case-control studies investigating associations of exogenous and endogenous factors with the risk of GC, mortality and morbidity from this disease. The literature search included articles published within the past 7 years, but significant findings from earlier publications relevant to the subject are

also mentioned in the review. In addition, the review addresses possible associations between GC and hereditary syndromes, genetic polymorphisms and occupational hazards. The majority of gastric malignancies are adenocarcinomas. Many meta-analyses differentiate between adenocarcinomas in the gastric cardia and non-cardia cancers. Therefore, unless otherwise specified, in this paper gastric cancer will refer to adenocarcinomas with specific localizations.

High-risk factors for GC

Diet

A study of dietary habits conducted in 191 patients with gastric cardia cancer, 190 patients with non-cardia cancer and 222 healthy controls established a statistically significant correlation between the risk of GC and dietary habits, including irregular meals, overeating and insufficient mastication: odds ratios (OR) were 4.2 (95% confidence interval (CI): 2.3–7.7), 4.7 (2.1–10.8) and 7 (1.3–5.3), respectively [4].

1. Meat consumption

A diet rich in meat (over 160 g/day) contributed to the cumulative risk of GC in the main group (obesity, high body mass index (BMI), consumption of hot tea and high-fat foods). First, the risk of GC was found to vary depending on the type of consumed meat. A direct (OR = 1.87 (95% CI: 1.01–3.47)) and negative (OR = 0.36 (95% CI: 0.19–0.68)) correlations were established between the risk of GC and the consumption of red and white meats, respectively. In the cited publication, beef, lamb, sausages, and hot-dogs were defined as red meat, whereas white meat referred to fish and poultry. Fish is rich in polyunsaturated fatty acids, therefore N-nitroso compounds are less likely to form as fish cooks; this prevents carcinogenesis [5]. Second, frying and charcoal grilling were associated with increased risk of GC due to the formation of carcinogens: OR 1.9 (95% CI: 1.0–3.6) and OR 1.8 (95% CI: 1.3–2.6), respectively [6]. Thus, excessive consumption of fried or grilled red meat that can potentially contain heterocyclic amines, N-nitroso compounds and polycyclic aromatic hydrocarbons is reliably linked to the risk of GC and increases the risk of colorectal cancer (CC) by 20–50% [7, 8]. Obviously, the risk of GC can be lowered by choosing a safer cooking technique and enriching the diet with nitrosation inhibitors, such as vitamins C, E, phenolic and other bioactive compounds extracted from fresh vegetables and fruits. For the European population, the lack of fresh vegetables and fruits in the diet is a significant factor promoting the risk of GC, similar to the consumption of smoked meat products (bacon, sausages and ham) [9]. Besides, excessive intake of cholesterol with animal source foods was correlated with the increased frequency of malignancies, including GC [10].

2. Excessive salt consumption

Although salt (sodium chloride) is important for normal metabolism, it has adverse systemic effects when ingested in excess. Sodium chloride stimulates secretion of gastric juice, thereby accelerating DNA synthesis and cell proliferation and leading to atrophic gastritis [9]. According to some researchers, the chronic form of this disorder may provoke GC. In other words, excessive salt consumption provokes GC. A meta-analysis of prospective cohort studies concluded that high and moderate salt intakes (as opposed to low intake of < 5 g/day) were significantly associated with elevated risk of GC: OR 1.68 (95% CI: 1.17–2.41) and OR 1.41 (1.03–1.93), respectively [11]. Another study conducted in 422 patients with GC and 649 community controls assessed the role of high-salt diet (corrected for the presence *H. pylori* infection, smoking status, tumor site and histological type) as an independent risk factor for GC. The study found that individuals who added salt at the table were at greater risk for GC (OR = 2.01 (95% CI: 1.16–3.46)) as early as within a year before the onset of cancer symptoms [12]. Two more systematic reviews provide convincing evidence that excessive salt consumption (> 5–6 g/day) is associated with elevated risk for GC [13, 14].

3. Pickles

Pickles are traditional components of many cuisines. They contain high amounts of preservatives, including salt, acetic and benzoic acid, diphenyls, and nitrates. Can pickles increase the risk of GC? Regular consumption of pickled vegetables in an East Asian population was associated with heightened risk of GC in comparison with the control group (no pickles in the diet). According to the meta-analysis, the cumulative OR was 1.52 (95% CI: 1.37–1.68); for case-control studies OR was 1.56 (95% CI: 1.39–1.75); for cohort studies, OR was 1.32 (95% CI: 1.10–1.59) [14]. Similarly, another publication reported a high risk of GC in individuals who included pickled vegetables in their diet (OR= 5.5 (1.4–19.5)) [15].

4. Nitrates

Nitrates accumulated in crops and drinking water (> 20 mg/L) negatively affect human health. Ingesting high amounts of nitrates was correlated with increased risk of GC and death from this disease [16].

5. Dietary fat

A study reported an association between GC and increased consumption of vegetable oil (OR = 4.5 (95% CI: 1.00–20.17); p = 0.03) and lard (OR = 1.4 (95% CI: 0.63–3.01) for the population of South-East Asia [17]. Perhaps, the specific effects of vegetable oils on carcinogenesis may be explained by their chemical composition. For example, the well-known Mediterranean diet, in which olive oil is the central ingredient, reduces the risk of some cancers. This effect is attributed to monounsaturated oleic acid, which inhibits the overexpression of the HER2 (*Her-2/neu*, *erbB-2*) oncogene; such inhibition is particularly important in breast cancer [18]. However, the intake of trans fats, including hydrogenated fish oil, is correlated with increased GC morbidity (p = 0.01) [19].

6. Regular coffee consumption

The effects of regular coffee consumption on the neoplastic transformation in the gastrointestinal tract are an interesting research object. The relative risk (RR) of GC was 0.94 (95% CI: 0.80–1.10) for individuals who drank 3–4 cups of coffee a day vs. RR = 0.93 (95% CI: 0.88–0.99) for those who drank 1–2 cups of coffee, in comparison with the control group (zero coffee consumption). After the correction by design, sex, duration of observation and population, a statistically significant difference was discovered between coffee consumption and diminished risk of GC (RR = 0.85 (95% CI: 0.77–0.95; case-control studies) [20]. However, the opposite results were generated by another analysis of subgroups stratified by sex, region and time, revealing increased risk for GC (RR = 1.36 (95% CI:

1.06–1.75)) [21]. Frequent, long-term coffee consumption is likely to be both a risk factor and an anti-risk factor for GC.

7. Hot meals and hot drinks

A case-control study included 600 cases of esophageal squamous-cell carcinoma (ESCC), 599 cases of gastric cardia carcinoma (GCA), 316 cases of gastric non-cardia adenocarcinoma (GNCA) and 1,514 controls. The risk of cancer rose by 150–219% in patients who had hot foods every day in comparison with those who rarely or never had their meals hot [22]. Another risk factor for GC was hot tea ($p < 0.05$) [23].

8. High intake of simple carbohydrates

Food products with a high glycemic index (GI) can increase the risk of cancer as they modulate the levels of insulin-like growth factor 1 (IGF1) associated with diabetes. High-carb diets were shown to be strongly associated with heightened risk of colon cancer and diabetes, but did not contribute to the incidence of GC [24].

Lifestyle

1. Alcohol and smoking

Regular smoking is recognized as a significant risk factor for GC in men (RR = 1.62 (95% CI 1.50–1.75)) and women (RR = 1.20 (95% CI: 1.01–1.43)). The risk for this cancer increases from 1.3 (for occasional smokers) to 1.7 for those who smoked 30 cigarettes a day; the long history of smoking raises the risk of gastric cardia and non-cardia cancers: RR = 1.87 (95% CI: 1.31–2.67) and 1.60 (95% CI: 1.41–1.80), respectively [25], with OR = 1.9 (95% CI: 0.85–4.50) [17].

A few publications reported the overall negative effect of alcoholic beverages on the development of GC. The meta-analysis of 75 studies [26] revealed that alcohol consumption was considerably associated with the risk of gastric non-cardia (OR = 1.19 (95% CI: 1.01–1.40); $p = 0.033$) and cardia cancers (OR = 1.6 (95% CI: 0.98–1.39); $p = 0.087$). The relative risk of GC for heavy beer/wine drinkers, in comparison with those who drank little alcohol, was 1.13 (95% CI: 1.03–1.24; $p = 0.012$) and 0.99 (95% CI: 0.84–1.16; $p = 0.857$), respectively [26]. When adjusted for smoking, education and BMI, the risk of GC was 2.00 (95% CI: 1.04–3.82) for regular alcohol drinkers (2–7 times a week) vs. those who consumed alcoholic beverages only occasionally (a few times a year); the risk for GC was 1.90 (95% CI: 1.13–3.18) for individuals consuming ≥ 100.0 g ethanol a week. The odds ratio for death from GC for men who consumed ≥ 0.5 L vs. < 0.5 L of alcohol per occasion was 2.95 (95% CI: 1.30–6.68) [27]. High alcohol consumption (>60 g/day vs. 0.1–4.9 g/day) was associated with increased mortality from GC (1.65; 95% CI: 1.06–2.58). Beer consumption over ≥ 30 g of alcohol/day was associated with increased GC morbidity (1.75 (95% CI: 1.13–2.73)); however, there was no significant association with wine or liquor consumption [28].

Thus, the risk of GC was minimal or zero for individuals who consumed moderate amounts of wine. A possible explanation is that extractives contained in wine (like the polyphenolic compound resveratrol) exert a broad spectrum of favorable effects: antioxidant, anti-inflammatory and anti-carcinogenic [29].

2. Opium consumption

A 4-year-long prospective cohort study was carried out in 50,045 participants, of whom 17% were long-term opium users with an average history of opium smoking or ingestion of 12.7 years. The study found that the risk of death from gastrointestinal cancer (GIC) was 1.55 (95% CI: 1.24–1.93) for all subjects. During the observation period, 387 people died of GIC; cancer-associated mortality in the group of opium users was 2.21 times higher (95% CI: 1.57–3.31) and also dose-dependent [30]. Other authors report an association between opium use and elevated risk of cardia and non-cardia adenocarcinomas (OR = 3.1 (95% CI: 1.9–5.1)). Similar to the previous cited study, they point to the dose-dependent effect (OR = 4.5 (95% CI: 2.3–8.5)) [31].

3. Sleep duration

The meta-analysis of 25 articles (a total of 1,550,524 participants and 86,201 GC cases) revealed that neither short nor long sleep duration (relative to the baseline value of 7 h) was associated with increased risk of cancer [32]. A prospective cohort study, which recruited 173,327 men and 123,858 women aged 51–72 years, reported a significant risk of death from GC in men (1.29 (95% CI: 1.05–1.59); $p = 0.03$) who normally slept 5–6 h vs. 7–8 h a day. By contrast, women who normally shad 5 h of sleep per day were at reduced risk of death from GC (0.76 (0.24–2.41)). It should be noted that the average weighted risk of other cancers did not significantly correlate with variations in sleep duration relative to the control group [33]; these findings were consistent with the results of other studies [34].

4. Chronic stress

There is a known psychosomatic link between the level of stress and gastritis (or gastric/duodenal ulcers) [35]; these conditions, together with co-existing inflammation, can predispose to neoplasms [36]. Stress aggravates gastric cancers; the underlying molecular mechanism of this phenomenon was studied in [37]. According the study, the expression of the β_2-adrenergic receptor (ADRB2) was elevated in gastric tumors and positively correlated with their size, stage and spread to lymph nodes. Induced by the stress hormone, the activation of the ADRB2 signaling pathway played the key role in the progression of cancer and metastasis. This suggests that GC progression may be regulated by the drugs for β_2 blockade (propranolol) as an adjunct to existing therapies [37].

Pharmacotherapy

1. Nonsteroidal anti-inflammatory drugs and aspirin

This class of drugs includes selective cyclooxygenase-2 (COX-2) inhibitors that, according to some studies, reduce the risk of GC and hold potential for chemoprevention [38]. Still, many aspects of their use, such as optimal dosing and therapy duration, remain understudied. Perhaps, the inhibitory effect of NAIDs on carcinogenesis stems from their ability to induce apoptosis of epithelial cells and regulate angiogenesis via COX-2-dependent and COX-2-independent signaling pathways [39]. A population case-control study enrolled individuals aged 30-79 years with esophageal adenocarcinoma ($n = 293$), esophageal squamous-cell carcinoma ($n = 221$), gastric non-cardia cancer ($n = 368$) and gastric cardia cancer ($n = 261$). The control group comprised 695 participants. Prolonged aspirin therapy over the course of 2 to 5 years reduced the risk of such cancers: OR = 0.37 (95% CI: 0.24–0.58), 0.49 (95% CI: 0.28–0.87), 0.46 (95% CI: 0.31–0.68), respectively, in comparison with the

control group (no aspirin), except cardia cancer (OR = 0.80 (95% CI: 0.54–1.19)) [40].

2. Statins

The association between blood cholesterol levels and the risk of GC is debatable. Statins inhibit endogenous cholesterol synthesis and are traditionally used to treat metabolic disorders; in addition, they can exert anticancer activity [41]. The meta-analysis of 26 randomized control and 8 observational studies of over 7,000 GC cases demonstrated that statins reduced the risk of GC by an average of 30% (RR = 0.73 (95% CI: 0.58–0.93)) [42].

Chronic diseases

1. Gastroesophageal reflux disease

Many studies have established a significant association between GERD and the risk of gastric cardia cancer [43, 44]. In most studies, GERD was associated with a 2- to 5-fold increase in GC morbidity. At the same time, some studies reported the lack of or the negative association between GERD and non-cardia gastric cancer [43–45].

2. Metabolic syndrome

Disrupted metabolism may be an additional risk factor for different cancer types and affect the overall survival of cancer patients. A retrospective study analyzed the clinical and histological data of 808 patients with GC and a history of metabolic syndrome (MS). The control group consisted of 1,146 individuals. Main group patients had high blood levels of triglycerides ($p = 0.007$), lower levels of high-density lipoproteins (HDL) ($p < 0.001$), a higher frequency of hypertension disease ($p < 0.001$) and diabetes (OR = 1.86 (95% CI: 1.39–2.48)). MS was associated with poorly differentiated gastric carcinoma and late progression to advanced stages according to the TNM classification [46].

Type 2 diabetes mellitus is the most common endocrine disorder characterized by hyperglycemia due to deficient insulin secretion and impaired metabolism. A few clinical studies investigated a causal link between diabetes and cancer. At least two studies showed that patients with diabetes mellitus were at greater risk for hepatic, pancreatic, gastric, colon, renal and breast cancers [47, 48]. According to a prospective cohort study, there was an association between early GC onset and hyperglycemia ($p = 0.000$; OR = 1.066), insulin resistance ($p = 0.024$; OR = 1.084), glycated hemoglobin (HbA1c) levels ($p = 0.004$; OR = 3.225), and low total blood cholesterol ($p = 0.005$; OR = 1.015). Besides, there was no significant association between the risk of early GC onset and the levels of the insulin-stimulated hormone adiponectin in the blood [49]. Hyperglycemia (glucose concentrations ≥ 5.3 mmol/L) contributed to the risk of GC associated with *H. pylori* infection [50]. It was discovered that HbA1c concentrations ≥ 6.0% (42 mmol/L) adjusted for sex, age and *H. pylori* seropositivity were a statistically significant factor predisposing to GC [50]. Likewise, an association was confirmed between the poor survival of GC patients ((1.73 (95% CI: 1.08–2.79) and the risk of death from gastric cardia cancer (3.40 (95% CI: 1.45–7.97)) in the setting of type 2 diabetes mellitus. HbA1C concentrations ≥ 6.0% (42 mmol/L) were the endogenous marker of increased mortality from GC (1.68 (95% CI: 1.07–2.63)) [51].

There is no firm association established between the levels of blood cholesterol and the risk of GC because the data generated by case-control vs. cohort studies are conflicting [52]. Nevertheless, high cholesterol should not be ignored if a patient is exposed to other risk factors for GC. The multivariate analysis of variance suggested a statistically significant association between the risk of gastric dysplasia (corrected to age and sex) and the levels of glucose of 100–125 mg/100 ml (RR = 2.261; 95% CI: 1.147–4.457); total cholesterol ≥ 240 mg/200 ml (RR = 6.299; 95% CI: 1.277–31.076); LDL of 130–159 mg/100 ml (RR = 0.250; 95% CI: 0.069–0.903), and MS (RR = 2.177; 95% CI: 1.082–4.379) [53].

3. Obesity

Recently, obesity has become a public health priority due to the growing incidence of cancers reliably associated with this condition. Globally, obesity-associated malignancies account for 11.9% of cancers in men and 13.1% of cancers in women. There is evidence that excess body weight may increase the risk of 13 different cancers, including endometrial, esophageal, renal, pancreatic, hepatocellular, gastric cardia, colorectal, ovarian, thyroid, bladder, and postmenopausal breast cancers meningiomas and multiple myelomas [54]. It is emphasized that abdominal obesity is a significant risk factor for GC [52, 55–57]. After adjustment for age, alcohol consumption, smoking, family history and total blood cholesterol, BMI from 27.5 to 29.9 was associated with the risk of grade 3 gastric dysplasia in men (OR = 1.87; 95% CI: = 1.24–2.81) and women (OR = 2.72; 95% CI: 1.44–5.16). For men with BMI from 27.5 to 29.9, the risk of developing gastric cardia dysplasia was OR = 1.78 (95% CI: 1.02–3.10); for BMI ≥ 30.0 OR was 2.54 (95% CI: 1.27–5.08); for women with BMI of 27.5–29.9 OR was 2.88 (95% CI: 1.27–6.55) and for women with BMI ≥ 30.0 OR was 2.77 (95% CI: 1.36–5.64) [52]. The analysis of 2,130 cancer cases from the sample of 913,182 patients showed that obesity increased the risk of gastroesophageal cancer and GC by 49–68% and 33–48%, respectively [57].

4. Autoimmune disorders

Autoimmune disorders may be regarded as an alternative etiological factor for chronic inflammation of gastric mucosa, promoting the risk of carcinogenesis. A systematic review of 52 observational studies discovered an association of some autoimmune diseases with the risk of GC (OR = 1.37; 95% CI: 1.24–1.52) [58]. Specifically, a significant link was established between GC and the following disorders: dermatomyositis (OR = 3.69; 95% CI: 1.74–7.79), pernicious anemia (OR = 2.84; 95% CI: 2.30–3.50), Addison's disease (OR = 2.11; 95% CI: 1.26–3.53), dermatitis herpetiformis (OR = 1.74; 95% CI: 1.02–2.97), IgG4-related disease (OR = 1.69; 95% CI: 1.00–2.87), primary biliary cholangitis (OR = 1.64; 95% CI: 1.13–2.37), type 1 diabetes mellitus (OR = 1.41; 95% CI: 1.20–1.67), systemic lupus erythematosus (OR = 1.37; 95% CI: 1.01–1.84) and Graves' disease (OR = 1.27; 95% CI: 1.06–1.52) [58].

Infection

1. Helicobacter pylori

Corrected for other risk factors, *Helicobacter pylori* infection has a critical role in the etiology and early onset of GC [59]. Patients seropositive for *H. pylori* and prone to excessive salt consumption were at a 10 times higher cumulative risk for GC than the control group (no antibodies to *H. pylori* and low-salt diet). *H. pylori* infection was shown to aggravate GC prognosis

in patients with a family history of cancer and smokers [60]. Interesting observations were described in a study that reported an association between the Lewis antigen system and the risk of GC [61]. The frequency of the Lea^+b^- phenotype was higher in patients with GC and *H. pylori* infection; the risk of GC was 3.15 times higher in the carriers of this phenotype than in those with the Lea^-b^+ phenotype [61]. Another meta-analysis that summarized the data generated by 22 studies demonstrated that patients who had undergone *H. pylori* eradication therapy were at lower risk for GC than those who had not (0.53; 95% CI: 0.44–0.64). Eradication of *H. pylori* ensured a stable therapeutic effect for asymptomatic infected individuals (0.62; 95% CI: 0.49–0.79) and patients who had undergone the endoscopic resection of GC (0.46; 95% CI: 0.35–0.60) [62].

2. Human papillomavirus

There are causal links between human papillomavirus (HPV) infection and GC. The meta-analysis of 30 studies (1,917 cases and 576 controls) found that the prevalence of HPV among the patients with GC was 28.0% (95% CI: 23.2–32.7; $p < 0.001$) and established an association between the infection and the risk of GC (OR = 7.388; 95% CI: 3.876–14.082; $p = 0.004$). According to the analysis of 15 case-control studies, HPV 16 was diagnosed in patients with GC 3 times more often than HPV 18. The researchers concluded that HPV may play a role in the pathogenesis of GC; more solid evidence can be obtained by isolating HPV from precancerous cells of gastric dysplasia lesions or and adenomas [63].

3. Epstein–Barr virus

About 90% of the population are infected with the Epstein–Barr virus (EBV). The virus was isolated from a variety of tumors, including nasopharyngeal and gastric cancers, Burkitt, Hodgkin and non-Hodgkin lymphomas. Today EBV infection is thought to be a potential risk factor for cancer. A correlation was established between the seropositivity for EBV and the nasopharyngeal cancer/Hodgkin lymphoma [64]. However, only 7–10% of gastric tumors were associated with EBV [64]; according to the authors of the analysis, this might be due to small sample sizes. For example, seropositivity for EBV was not associated with elevated risk of GC in the main and control groups that comprised 185 and 200 cases, respectively. High antibody titers for the Epstein–Barr nuclear antigen were associated with longer survival in patients with cardia cancer [65]. In another retrospective study (54 individuals with gastric adenocarcinomas), the risk of cancer in patients seropositive for IgA against the viral capsid protein and IgG against the early antigen R-component was 4 and 2 times higher, respectively, than in the control group. Antibody titers against EBV were significantly higher in patients who were later diagnosed with EBV-associated GC than in those with GC not associated with EBV infection [66].

These findings suggest that the failure of the immune system to control EBV infection may increase the risk of malignancies in the long term [66]. According to the published study of the associations between GC and a coinfection with 3 pathogens (*H. pylori*, HPV and EBV) [67], the GC specimens contained the nucleic acids of *H. pylori*, EBV and HPV in 87, 20 and 3% of cases, respectively. *H. pylori* was mainly represented by the $cagA^+$ (*H. pylori* - $cagA^+$) strain. The cagA gene encodes the virulence factor, which is essentially an oncogenic protein capable of causing hyperplasia of the gastric epithelium and polyposis. A coinfection with *H. pylori-*$cagA^+$ and EBV was correlated with advanced stages of GC, and the presence of EBV infection was correlated with distant metastasis [67]. Consequently, measures for *H. pylori* and EBP prevention help to ward off GC and especially its aggressive forms.

Ionizing radiation

The literature analysis shows that the association between the risk of GC and ionizing radiation doses remains understudied. Exposure to both natural or man-made sources of radiation (accidents at nuclear power stations) can cause multiple damage to human genes and induce shifts in the global gene expression [68].

Some secondary tumors can be provoked by radiation therapy for the abdomen. The cumulative coefficient of primary GC incidence in the studied group (22,269 subjects) was 1.45% 30 years after the diagnosis. Individuals who received radiation therapy for testicular cancer were at a 6-times higher risk of developing GC (OR = 5.9; 95% CI: 1.7–20.7). The risk grew with the total dose approaching 50 Gy ($p < 0.001$), OR = 20.5 (3.7–114.3) in comparison with the total dose of <10 Gy. Thus, the highest risk of developing secondary cancers was observed for the total radiation dose of >30 Gray [69]. It should be noted that in its latent state, EBV associated with GC expresses a very small number of genes. However, exposure to ionizing radiation leads to the NF-κB-mediated activation of the lytic form of the virus, whose persistence is an additional risk factor for GC [70].

Occupational hazards

Exogenous factors predisposing to GC include social factors and occupational hazards. For example, an association was discovered between the heightened risk of GC quantitatively expressed as the relative indexes of inequality and a few social factors [71], such as low educational status (2.97 (95% CI: 1.92–4.58)), job (4.33 (95% CI: 2.57–7.29)), socioeconomic status (SES) (2.64 (95% CI: 1.05–6.63)), and income (1.25 (95% CI: 0.93–1.68)). Differences in GC incidence between social groups were more pronounced in another study [72] showing that the risk of GC decreased from 22.7% to 2% ($p < 0.001$), from 12% to 0.5% ($p < 0.001$) and from 6.5% to 0.1% ($p < 0.001$) in the groups with low, moderate and high SES. A significant correlation was observed between low SES and GC incidence and mortality [73]. According to the meta-analysis of 25 studies (9,773 GC cases and 24,373 controls), the risk of GC decreased in groups with a high educational status: OR and the relative index of inequality were 0.60 (95% CI: 0.44–0.84) and 0.45 (95% CI: 0.29–0.69), respectively [73].

Stratification of occupational hazards in a Swedish population revealed an almost two-fold difference in the risk of GC between different socio-economic groups [74]. Individuals involved in manual labor (miners, quarry workers, fishermen, construction workers, packers, loaders, warehouse workers, clerical workers, nurses and postmen) were at higher risk for GC [74]. Standardized incidence ratios of gastric cardia cancer were significantly increased for male gardeners, transport workers, chemical industry workers and bricklayers. Cement and mineral dusts were the main occupational risk factor for GC [74].

In a Spanish population, the risk of developing GC was statistically significant for male cooks (OR = 8.02), wood processing plant operators (OR = 8.13), food and related product machine operators (OR = 5.40), miners and quarry workers (OR = 4.22; 95% CI: 0.80–22.14)) [75]. The risk of GC was also significant for men and women involved in plant

cultivation and exposed to pesticides (OR = 10.39; 95% CI: 2.51–43.02), as well as for those involved in manufacturing and exposed to asbestos (OR = 3.71; 95% CI: 1.40–9.83) and wood dust (OR = 3.05) [75].

Cr(VI) is an established carcinogen provoking lung cancer. The meta-analysis of 56 cohort and 74 case-control studies sought to test the hypothesis about the association between the risk of GC and occupationally inhaled chromium in chrome plating and leather workers and those exposed to Portland cement [76]. The cumulative relative risk was 1.27 (95% CI: 1.18–1.38); in comparison with other studies reporting the increased risk for lung cancer, RR for GC was 1.41 (95% CI: 1.18–1.69) [76]. On the whole, these results allow identifying Cr(VI) as a risk factor for GC.

Genetic factors for GC: hereditary cancer syndromes and genetic polymorphism

1. Hereditary GC syndromes and family history

The family history of GC is another factor that augments the risk of the disease 1.5–3.5 fold if at least one first-degree relative has GC [77]. Although GC is mostly sporadic, familial aggregation is observed in about 10% of cases and 1–3% of cases are associated with cancer syndromes [78, 79]. According to a study, the incidence of GC was higher in individuals whose relatives had a history of early-onset GC (before 50 years) [80, 81]. The frequency of GC was higher among patients whose first-degree relatives had GC (OR = 2.7; 95% CI: 1.7–4.3). If two or more relatives had GC, OR rose to 9.6 (95% CI: 1.2–73.4) [82]. The incidence of GC was also higher in patients whose first/second degree relatives had a history of malignancies including GC, breast or lung cancer, gynecological and hematologic cancers, as shown by the long-term observations of the main group (n = 44; 54.5%, $p < 0.01$) and the control group (n = 44; 11.4%, $p < 0.01$) [79]. It is reported that GC-associated mortality was higher in patients with a family history of *H. pylori* and GC (OR = 8.2; 95% CI: 2.2–30.4) than in the control group (no family history of *H. pylori* and GC). At the same time, non-cardia cancer was the most common malignancy in the sample [83].

The most significant hereditary cancer syndrome manifested as GC is hereditary diffuse gastric cancer (HDGC). This syndrome is associated with pathogenic variants of the *CDH1* gene, which encodes the cell adhesion protein E-cadherin. A study conducted in 75 families found that the cumulative risk of GC was 70% and 56% for female and male carriers of the pathogenic *CDH1* variants, respectively, by the age of 80 years [84]. An earlier study involving 13 families produced the opposite results: the cumulative risk of GC was 67% for men and 83% for women [85]. It should be noted that the cited study included 3 Maori and one Pakistani families. Thus, ethic differences should be accounted for when estimating the cumulative risk of HDGC. Besides, both publications show that female carriers of the pathogenic *CDH1* alleles are at increased risk for lobular breast cancer (cumulative risk of 39–42% by age of 80 years). Importantly, the pathogenic CDH1 variants are detected in only 40% of patients with clinical signs of HDGC. Genetic causes of this disease in other patients are obscure [86].

Another hereditary cancer syndrome contributing to the risk of GC is the Peutz–Jeghers syndrome. It is characterized by the development of gastrointestinal hamartomatous polyps. Its distinctive feature is the presence of melanin spots on the lips, buccal mucosa and other parts of the body. The disease is manifested as gastrointestinal tumors, including GC. The affected women are at increased risk for breast cancer. The disease is caused by the pathogenic variants of the *STK11* gene [87]. According to some estimates, the cumulative risk of GC in patients with the Peutz–Jeghers syndrome aged 15 to 64 years is 29% [88].

Another syndrome that significantly increases the risk of GC is juvenile polyposis. This condition is caused by the pathogenic mutations in the *SMAD4* or *BMPR1A* genes. As a rule, juvenile polyposis affects children but can also arise at older age. The cumulative risk of GC is 21% for patients afflicted with this syndrome [89].

Among other hereditary cancer syndromes that aggravate the risk of GC are Lynch syndrome, Li–Fraumeni syndrome, familial adenomatous polyposis, MYH-associated polyposis, gastric adenocarcinoma and proximal polyposis of the stomach [86]. There is evidence that patients with ataxia-telangiectasia, Bloom syndrome, Cowden syndrome, and xeroderma pigmentosum are at increased risk for GC [89].

Another condition worth mentioning is the syndrome of hereditary breast and ovarian cancers associated with mutations in the *BRCA1* and *BRCA2* genes. Carriers of the pathogenic *BRCA1/BRCA2* alleles are at increased risk for GC [90, 91]. Although this risk is only slightly increased, the syndrome is very common and therefore its association with GC may be clinically significant.

In addition to the listed hereditary cancer syndromes (see Table), the risk of GC is elevated in patients with inherited primary immunodeficiency [92]. Recently, the incidence of GC among such patients has started to decline; this might be tied to the spread of *H. pylori* eradication therapy [93].

2. Genetic polymorphism

It is not only the pathogenic variants of nucleotide sequences associated with cancer syndromes that contribute to the risk of developing GC, but also non-pathogenic populational polymorphisms. According to one of the largest research studies of twins conducted in Sweden, Denmark and Finland, the contribution of genes to GC is much greater than to other nosologies. The risk of GC for a male monozygotic twin of a twin with GC was 9.9 times higher than for a male monozygotic twin of a twin without GC. Concordance for GC in male monozygotic twins was 0.08, i.e. there is an 8% probability of GC in one of the twins if the other already has GC [94].

According to a 2019 meta-analysis that covered 186 studies, the strongest associations were observed for 9 variants of 9 genes: *APE1* rs1760944, *DNMT1* rs16999593, *ERCC5* rs751402, *GSTT1* 0/0 genotype, *MDM2* rs2278744, *PPARG* rs1801282, *TLR4* rs4986790, *IL-17F* rs763780 and *CASP8* rs3834129. The metanalysis included a total of 61 gene variants [95]. The strongest association with GC was shown for the G allele of the *APE1* gene (rs1760944): OR was 1.77 [95]. The existing data on the associations between genetic polymorphisms and GC are not clinically relevant and cannot be used to elaborate screening recommendations. So, it is more reasonable to focus on the family history while estimating the risk of GC.

Factors reducing the risk of GC

Fruits and vegetables

By and large, diets enriched in fruits and vegetables (especially fresh) were negatively correlated with the risk of GC [4, 9]. Regular intake of fruits and vegetables reduced the risk of GC by

Table. Hereditary cancer syndromes associated with increased risk of GC

Syndrome	Genes	GC risk	Inheritance pattern	Reference
Hereditary diffuse GC	*CDH1*	56–83%	Autosomal-dominant	[84, 85]
Peutz–Jeghers syndrome	*STK11*	29%	Autosomal-dominant	[86]
Juvenile polyposis	*SMAD4, BMPR1A*	21%	Autosomal-dominant	[86]
Lynch syndrome	*MLH1, MSH2, MSH6, PMS2, EPCAM*	1–13%	Autosomal-dominant	[86]
Li–Fraumeni syndrome	*TP53*	2,8%	Autosomal-dominant	[86]
Familial adenomatous polyposis	*APC*	1–2%	Autosomal-dominant	[86]
Hereditary breast and ovarian cancers	*BCRA1, BRCA2*	Increased	Autosomal-dominant	[86]
MYH-associated polyposis	*MYH*	Increased	Autosomal- recessive	[86]
Gastric adenocarcinoma and proximal polyposis	Pathogenic variant of *APC* promoter	Increased	Autosomal-dominant	[86]
Ataxia-telangiectasia	*ATM*	Likely increased	Autosomal- recessive	[89]
Bloom syndrome	*BLM*	Likely increased	Autosomal- recessive	[89]
Cowden syndrome	*PTEN*	Likely increased	Autosomal-dominant	[89]
Xeroderma pigmentosum	*DDB2, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, POLH, XPA, XPC*	Likely increased	Autosomal- recessive	[113]

48–70% and 46–68%, respectively [22], which was consistent with the findings of another research study (OR = 0.3; 95% CI: 0.1–1.0) [6]. By contrast, low intake of fruits and vegetables promoted the risk of GC (OR = 1.2; 95% CI: 0.74–1.96) [17]. Onions and garlic had a protective effect on the gastrointestinal tract and reduced the risk of GC [96]. A negative association was established between the risk of GC and consumption (often vs never) of garlic stalks (OR = 0.30; 95% CI: 0.12–0.77). In another study, increased consumption of allium vegetables (onions, garlic, leeks, shallots, garlic stalks, Chinese chives, Welsh onions) reduced the risk of GC (OR = 0.54; 95% CI: 0.43–0.65) [97]. The meta-analysis of 18 studies showed that the relative risks of developing colorectal cancer and GC were 0.69 (95% CI: 0.55–0.89) and 0.53 (95% CI: 0.31–0.92), respectively, in the main group (garlic consumption > 28.8 g/week) in comparison with the control group (3.5 g/week) [98].

Dietary fiber intake

Dietary fiber is a food component that is poorly digested by the gastrointestinal tract of humans but can be fully digested by the intestinal microbiota. A systematic review [99] analyzed 21 publications, to find that the odds ratio of GC for high dietary fiber intake was 0.58 (95% CI: 0.49–0.67; $p < 0.001$). Moreover, inclusion of 10 g of dietary fiber in the diet was associated with a 44% reduction in the risk for GC [99].

Tea

Similar to coffee, regular tea consumption was also studied for the association with GC. Polyphenolic compounds contained in tea exert antioxidant activity and have a variety of anticancer effects: they inhibit nitrosation and stimulate apoptosis in carcinoma cell lines. About half of the of prospective cohort studies investigating the effect of regular tea consumption on GC did not find any associations between green tea consumption and GC, whereas the rest established a negative association [100].

Dietary carotenoids

Intake of α-, β-carotins, lycopene, and lutein was negatively correlated with the risk of GC: OR = 0.59 (95% CI: 0.37–0.92); 0.52 (95% CI: 0.46–0.59); 0.88 (95% CI: 0.55–1.41) and 0.85 (95% CI: 0.56–1.30), respectively. The RRs of GC at 95% CI were as follows: 0.72 (0.50–1.03), 0.79 (0.58–1.07), 0.80 (0.60–1.07) and 0.95 (0.77–1.18), respectively [101]. Thus, case-control studies established a negative correlation between the intake of dietary carotenoids and the risk of GC.

Vitamins

High vs. low vitamin intake was negatively associated with the risk of GC (RR: 0.78 (95% CI: 0.71–0.83)) [102]. However, if daily intake of vitamins was increased 4 times (9 studies), the risk of GC also increased slightly (OP = 1.20; 95% CI: 0.99–1.44) [102]. The analysis of dose-dependent effects of vitamin A (1.5 mg/day), vitamin C (100 mg/day) and vitamin E (10 mg/day) indicated a decline in the risk of GC by 29%, 26% and 24%, respectively [102]. Diets high in fruits (100 g/day) rich in vitamin C were negatively correlated with the risk of GC [9]. Interestingly, intake of food supplements containing garlic extracts, vitamins C, E and selenium was associated with reduced morbidity and mortality from GC although the associations were statistically insignificant. By contrast, vitamin therapy was significantly negatively correlated with mortality from esophageal cancer and GC (0.51; 95% CI: 0.30–0.87; $p = 0.014$) [103].

Vitamin D, the precursor of the steroid hormone calcitriol, regulates a number of metabolic and signaling pathways in the cells. Low blood levels of vitamin D were shown to correlate with cancer [104]. Spanish patients with GC had low blood concentrations of vitamin D, in comparison with the control group (no cancers and vitamin D deficiency): OR = 8.8 (95% CI: 5–22; $p < 0.0001$) [105]. Case-control studies conducted in the USA demonstrated that both deficiency (< 20 ng/L) and excess (20–29 ng/L) of vitamin D were far more common in patients ($n = 103$) with incomplete gastric metaplasia than in healthy individuals ($n = 216$) with vitamin D concentrations in the blood ranging from 30 to 100 ng/L; this factor might play a role in the development of gastric adenocarcinoma *in situ* [106]. Sufficient concentrations of vitamin D (over 20 ng/L) in the blood plasma of Korean adults were associated with high efficacy of *H. pylori* eradication therapy and low risk of GC (OR = 0.57; 95% CI: 0.32–1.00) [107].

The link between dietary folic acid (vitamin B9) and GC remains understudied. Studies in mice with induced gastric dysplasia demonstrate that dietary folic acid slows DNA

hypomethylation in the epithelial cells and stromal myofibroblasts of the stomach associated with worse survival [108]. Besides, folic acid exerts an inhibitory effect on inflammation [108]. Still, the efficacy of folic acid in preventing and treating gastric malignancies is yet to be proved in future research.

Exercise

Some systematic reviews indicate that regular exercise and sports are usually negatively correlated with the development and relapse of cancer. For example, exercise was associated with a 20–50% reduction in the risk of lung [109] and breast [110] cancers. The cited reviews discussed a few possible causes underlying this phenomenon: optimization of hormonal status, reduction of oxidative stress in tissue due to oxygen saturation and activation of immune mechanisms. Four studies demonstrated that exercise had a protective effect against gastric cardia cancer (OR = 0.80; 95% CI: 0.63–1.00), and 5 studies showed the same effect against non-cardia cancer (OR = 0.63; 95% CI: 0.52–0.76), regardless of sex, study quality, study design, and geographic location [111].

CONCLUSION

Based on the prevailing risk factors for GC described in the review, a few cancer prevention strategies can be singled out, including measures for reducing the risk of primary gastric malignancies, prediction of GC risk using genotyping panels of genetic markers and early detection. Obviously, by changing modifiable behaviors (quitting smoking, reducing salt consumption to <5 g/day according to WHO recommendations, enriching the diet with vegetables, fruits, dietary fibers and antioxidants) one can significantly reduce the risk of developing GC. Special attention should be paid to the detection and treatment of *H. pylori*, which is the primary infectious factor of GC. Eradication therapy for *H. pylori* in patients with diagnosed GC reduces the risk of metachronous recurrence by almost 50% [112].

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ENDOTHELIAL DYSFUNCTION IN COVID-19 PATIENTS AND CLINICAL APPLICATION OF LASER THERAPY

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This review covers the published papers describing endothelial dysfunction pathogenesis and molecular mechanisms behind the effect of low-level laser therapy on regulation of the said pathogenesis. Herein, we present the current experience of using laser therapy to prevent development of endothelial dysfunction in the context of post-COVID-19 rehabilitation, as well as the accumulated data on the methods of combination of external or intravenous laser blood therapy and influence on the immunocompetent. We provide justification for practicing personalized approach at various stages of post-COVID-19 rehabilitation and treatment. The basis allowing greater efficacy of post-COVID-19 rehabilitation, including protocols making use of laser therapy, is the analysis of single-nucleotide polymorphisms of genes that determine adaptation processes, peculiarities of the immune response to infectious pathogens, predisposition to the development of respiratory distress syndrome, severe pneumonia, sepsis, multiple organ failure, development of endothelial dysfunction, thrombotic complications, the analysis that allows identification of patients running higher risk of critical conditions.

Keywords: COVID-19, endothelial dysfunction, rehabilitation treatment, laser therapy, personalized approach, genotyping

Author contribution: AV Kochetkov — idea, data analysis and interpretation; NYu Ponomareva — literature analysis, manuscript drafting; NG Kadnikova — laser therapy technique application, data collection; VG Mitkovskij — research organization task setting; EN Yampolskaya — research results analysis and interpretation; VV Lazarev — selection of patients for application of the technique, analysis of the results.

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ЭНДОТЕЛИАЛЬНАЯ ДИСФУНКЦИЯ У БОЛЬНЫХ COVID-19 И КЛИНИЧЕСКОЕ ПРИМЕНЕНИЕ ЛАЗЕРНОЙ ТЕРАПИИ

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В обзоре литературы по патогенезу развития дисфункции эндотелия и молекулярным механизмам влияния низкоинтенсивного лазерного излучения на его регуляцию представлены актуальный опыт применения лазерной терапии для предотвращения развития эндотелиальной дисфункции в реабилитации больных COVID-19, методы сочетания наружного или внутривенного лазерного освечивания крови и воздействия на иммунокомпетентные органы. Обоснован персонализированный подход к лечению и профилактике на различных этапах реабилитации пациентов, перенесших COVID-19. Анализ однонуклеотидных полиморфизмов генов, детерминирующих процессы адаптации, особенности иммунного ответа на инфекционные возбудители, предрасположенность к развитию респираторного дистресс-синдрома, тяжелому течению пневмонии, сепсиса, полиорганной недостаточности; развитию эндотелиальной дисфункции, тромботическим осложнениям для выявления пациентов с повышенным риском критических состояний является основой повышения эффективности восстановительного лечения таких больных, в том числе с применением методов лазерной терапии.

Ключевые слова: COVID-19, эндотелиальная дисфункция, восстановительное лечение, лазерная терапия, персонифицированный подход, генотипирование

Вклад авторов: А. В. Кочетков — идея, анализ и трактовка данных; Н. Ю. Пономарева — анализ литературы, подготовка черновика рукописи; Н. Г. Кадникова — применение методики лазерной терапии, сбор данных; В. Г. Митьковский — постановка задачи организации исследования; Е. Н. Ямпольская — анализ и трактовка результатов исследования; В. В. Лазарев — отбор пациентов для применения методики, анализ результатов.

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The global COVID-19 pandemic caused by the SARS-CoV-2 coronavirus is a challenge for the entire mankind, but the first to search for solutions thereto are scientists and doctors that are tasked with finding ways to curb incidence, effectively treat and rehabilitate COVID-19 patients and minimize the associated complications and mortality.

One of the many features of COVID-19 is the pronounced non-specificity of the observed pathological processes and its capacity to damage both organs and tissues and functional regulatory systems. At the same time, development of endothelial dysfunction can be named a factor that largely unites various disorders. There is an opinion gaining popularity that vascular endothelial damage is the cornerstone of organ dysfunction in severe SARS-CoV-2 infection cases [1].

The patients that died from respiratory failure resulting from COVID-19 had diffuse alveolar injury with perivascular T-cell infiltration as a specific histological pattern registered in the peripheral lung. The lungs of these patients have distinctive vascular features: severe endothelial damage associated with intracellular presence of the virus and fragments of destroyed cell membranes. Histological analysis of pulmonary vessels of COVID-19 patients revealed widespread thrombosis and microangiopathy. COVID-19 patients had alveolar capillary micro clots 9 times more often than influenza patients ($p < 0.001$). All this indicates that the disease also translates into a severe endothelial dysfunction [2].

Endothelial dysfunction (EnD) — a complex multifaceted process typically seen in the context of cardiovascular,

metabolic and immune disorders — is a current and serious challenge for clinical practitioners, even when considered outside of connection to COVID-19 [3]. And with a developing viral infection in the background, exploring the means to prevent this pathology is of paramount importance.

Endothelium is a cardiovascular endocrine organ that, in critical situations, enables communication between blood and tissues [4]. It acts as a barrier between the blood and the vascular wall, helps adaptation to changing environmental conditions through local regulation of vascular tone, vascular wall integrity protection etc. Normally, endothelial cells, following alterations in blood flow rate, exposure to mediators or neurohormones, react by increasing the synthesis of substances that cause relaxation of the vascular wall's smooth muscle cells (nitric oxide (NO) and other relaxants). They also work to prevent thrombogenesis by blocking platelet aggregation, oxidating low density lipoproteins, expressing adhesion molecules, "sticking" monocytes and platelets to the vascular wall, producing endothelin etc. Compensatory mechanisms are activated under the influence of a damaging factor. In case of prolonged exposure to such a factor (hypoxia, intoxication, inflammation, hemodynamic overload, etc.), compensation fades and a pathological process develops. Endothelial dysfunction is an imbalance between biologically active substances synthesized by endothelial cells (potentially protective NO, endothelial hyperpolarization factor, prostaglandins) and damaging substances (endothelin-I, thromboxane A2, superoxide anion etc.) [5].

It is the genotype that shapes all these normal and pathological molecular mechanisms of endothelium's adaptive response to normal or excessive influences. Currently, there are over 1500 genes established to have an association with multifactorial human diseases.

The human genome investigation efforts in the context of the Human Genome Project, Hap Map project, The 1000 Genomes projects, The SNP Consortium, have revealed mutations and single nucleotide polymorphisms (SNP) in genes encoding protein molecules of the body's regulatory systems. Their associations with various pathologies were either confirmed or refuted [6–9].

For example, the public Online Mendelian Inheritance in Man database (OMIM) [10] and the single nucleotide polymorphisms database contain more than 3.5 million SNP markers [11].

One of the studies investigating the significance of polymorphism of various genes considered possible contributors to the development of cardiovascular diseases (CVD) identified 105 genes that most likely support pathophysiology of CVD [12]. The researchers focus on genes that determine endothelium's properties, its role in the development of local vasospasm/vasodilation, hemostasis, inflammation, atherosclerosis, angiogenesis, etc [13–14].

Long before the COVID-19 pandemic, significant individual characteristics of critical conditions observed dynamically triggered the analysis of the results of geno-phenotypic examinations of IC patients [15–19]. These studies presented comorbid conditions gene diagnostics, identified SNP markers associated with an increased risk of community-acquired and nosocomial pneumonia, risk of development of an acute respiratory distress syndrome, CVD-related thrombotic complications. The results of the analysis of identified gene polymorphisms the products of which shape regulation (hemostasis, renin-angiotensin system regulation, immune system regulation, i.e. individual response to infectious pathogens and production of cytokines, drug metabolism) provide justification to screening patients running a high risk of development of life-threatening conditions. Such patients need non-standard treatment approaches in critical situations.

Personalized approach is a strategy popular at various stages of rehabilitation. In particular, patients in cardio- and neurorehabilitation undergo genotyping enabled by various SNP panels [20–25]. In clinical practice, molecular markers of individual susceptibility to various patterns of CVD development (the most important of which is endothelial dysfunction) allow predicting sudden death of a patient or the development of catastrophic multiple organ complications, as well as choosing the most effective therapies, which may be pharmacotherapy and non-drug methods [26–27], including laser therapy.

It was observed that patients with different phenotypes respond to laser therapy differently. In particular, low level laser therapy (LLLT) was more effective in patients that exhibited domination of reactions of the sympathetic nervous system than in those whose parasympathetic responses were stronger [28]. The peculiarities of the endothelial function were found to be behind this difference. The said function is genetically determined by the cooperation of gene regulatory networks [26–27]; it needs to be studied further, same as the collation of geno- and phenotypical characteristics and individual responses to various therapies.

Concomitant diseases can synergistically activate pathophysiological pathways. Thus, inflammation activates vascular pathology through pro-inflammatory cytokines, endothelin-1 and nitric oxide, which contributes to long-term damage to fatty acids, proteins, DNA, and mitochondria. Dysfunctional energy metabolism (impaired production of mitochondrial ATP, the formation of amyloid-β), development of endothelial dysfunction and violation of the blood-brain barrier lead to the cerebral blood flow reduction and chronic cerebral hypoperfusion with oxygen and nutrient deficiency, metabolic and synaptic disorders, neurodegeneration and white matter atrophy, cognitive dysfunction and development of Alzheimer's disease [29]. Identification and assessment of the entire complex of pathogenetic mechanisms driving inflammation form basis for targeted therapies designed to remedy the reduced cerebral blood flow and hypometabolism.

Molecular-cellular and physiological mechanisms of vascular homeostasis regulation

The key manifestations of EnD are abnormal bioavailability of nitric oxide (NO), the main vasodilator, which results from suppression of endothelial NO synthase (NOS), with the NO synthesis decreasing consequently [30]. Under normal physiological conditions, there is a balance between vasoconstrictors secreted by the endothelium and vasodilators. Any violation of this balance leads to a local spasm and vascular tone growth. As a result, the compensatory capacity of endothelium deteriorates gradually, which translates into breakdown of a rather complex regulation of the natural vascular bed expansion and shrinking mechanisms [13]. Endothelium plays a key role in maintaining vascular homeostasis since it releases biologically active substances (Table 1), but is also susceptible to the effects of external regulators [31–33]:

– mast cells that release heparin and histamine;

– platelets containing vascular endothelial growth factors and blood coagulation factors, etc;

– hormones and neuropeptides (adrenaline, acetylcholine, histamine, bradykinin, etc).

Despite the fact that the regulation mechanisms are known (see Table 1), the ways to remedy endothelial dysfunction pharmacologically require further comprehensive study and

Table 1. Physiologically active substances, regulators of the circulatory system, synthesized in the endothelium

Vascular wall tone regulators	
Vasoconstrictors	Vasodilators
Endothelin I–II Angiotensin II Thromboxane (TXA_2) Prostaglandins H_2 and G_2	Nitric oxide (NO) Prostaglandin E_2 (PGE_2) Endothelial hyperpolarizing factor (EDHF) Bradykinin C-natriuretic peptide Adrenomedullin Endothelin III
Regulators of hemostasis and antithrombosis	
Prothrombogenic factors	Antithrombogenic factors
Platelet-derived growth factor (PDGF) tissue plasminogen activator inhibitor (PAI-I) Von Willebrand factor (coagulation factor VIII) Angiotensin IV Endothelin I	NO Tissue plasminogen activator (t-PA) Prostacyclin (PGI_2)
Leukocyte adhesion regulators	
Adhesion stimulants (E-selectin, P-selectin, intercellular adhesion molecule 1 (ICAM-I), vascular cell adhesion molecule 1 (VCAM-I)	
Vascular growth regulators	
Stimulants	Inhibitors of myocyte migration and proliferation
Endothelin-I Angiotensin-II Superoxide radicals Growth factors: fibroblast, platelet, insulin-like, transforming growth factor β (bFGF, PDGF, IGF, TGFβ)	NO Prostacyclin (PgI_2) C-natriuretic peptide
Regulators of inflammation, vascular permeability, apoptosis of vascular wall components	
Stimulants Tumor necrosis factor α (TNFα) Superoxide radicals (O_2^-, $OONO^-$) Protein kinase C	Inhibitors
	NO

evaluation, since currently they have negative side effects and deliver mediocre results [3]. Physiotherapeutic procedures are one of the options considered when the functional state of the endothelium needs to be normalized [34].

Primary and secondary mechanisms of the biomodulating action of low-level laser therapy (LLLT)

The current understanding of the biomodulating action of LLLT, which agrees with the clinical practice of laser therapy use, has the thermodynamic triggering of Ca^{2+} dependent processes as the primary mechanism. Once the various intracellular components have absorbed photon energy (laser light), the intracellular calcium store is activated, Ca^{2+} ions are released and the concentration in the form of two waves with half periods of 100 and 300 s is increased, which is followed by the cascade of responses on all levels, from cells to the entire body: activation of mitochondria, cellular metabolism and proliferation, normalization of the immune and vascular systems, inclusion of the autonomic and central nervous system into the process, etc (Fig. 1) [35–37].

Versatility and high efficacy of laser therapy, which is unique type of physiotherapy, relies on the action at cellular level, with the maximum frequency of optical band electromagnetic waves and laser light coherence (monochromaticity).

Influence of LLLT on the vascular homeostasis regulation factors and immunity

It is well known that almost all of the above regulators (Table 1) are, to a certain degree, associated with changes in Ca^{2+} concentration; we will cite only a few reviews [38–39].

From the point of view of the subject researched, we should be primarily interested in nitric oxide, the synthesis and release of which is Ca^{2+} dependent [40]; therefore, it is not surprising that many studies confirm that LLLT can stimulate the release of NO, thus enabling regulation of the vascular homeostasis [41–47].

Moreover, there are studies demonstrating a direct relationship between intracellular Ca^{2+} concentration increase and NO release intensity and subsequent vasodilation [48–50].

Endothelial system normalization in children with bronchial asthma was confirmed by changes in various parameters of blood plasma, including endothelin-1 and nitric oxide [51–52]. The capacity of LLLT to effectively stimulate the release of PGE_2 has been proven both in experimental [53–55] and clinical studies [56–58].

In arterial hypertension patients, regimens of both external laser therapy (ELT) pulsed infrared LLLT) and intravenous laser blood therapy (IVLBT) improve a number of biochemical, hemorheological and hormonal parameters (C-peptide, insulin, angiotensin, bradykinin, aldosterone, cortisol), and the improvements persist for at least 6 months [59–61].

Many researchers have shown the role of the kallikrein system in hemovascular regulation and the possibility of its correction through illumination of blood with red laser (wavelength of 635 nm) and/or incoherent ultraviolet (UV) light [62–65].

Current laser therapy techniques actively exploit the well-known anti-inflammatory effect of LLLT. Numerous studies have shown that LLLT can activate phagocytes (which absorb foreign particles of bacteria, viruses, and dying cells) and the synthesis of cytokines, including interferons (IFNs), which spearhead the first line of defense against viruses

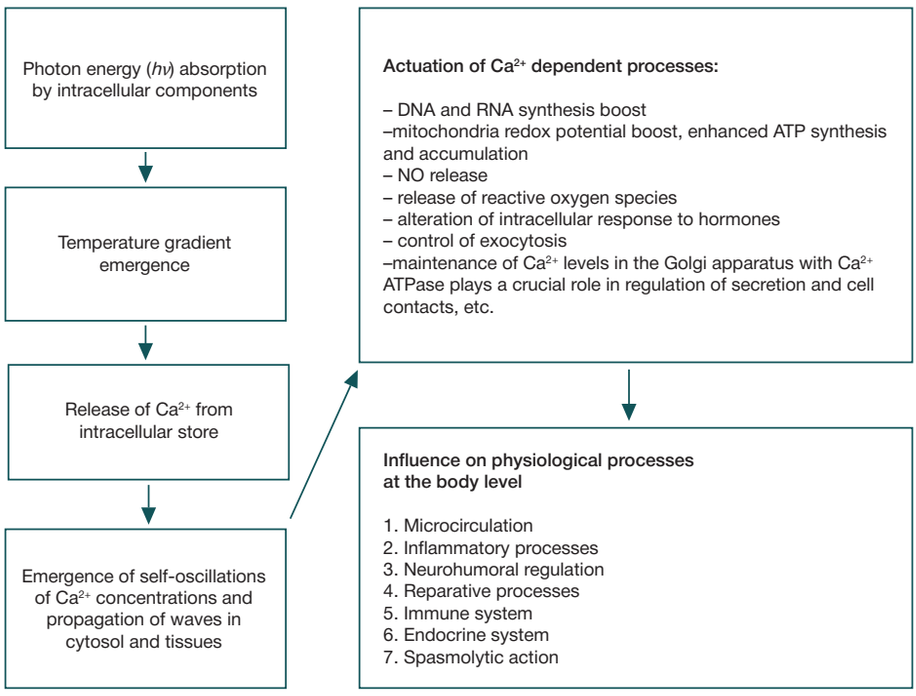

Fig. 1. Molecular-cellular mechanisms of the biomodulating action of LLLT

and contribute greatly to the development of adaptive immunity. IFNα and IFNβ, which are secreted by lymphocytes, macrophages, fibroblasts and some epithelial cells, stimulate the activity of macrophages and natural killer cells (NK). IFNγ, secreted by T-cells and EK, regulates the immune response, has antiviral and antitumor effects. In addition, LLLT improves micro- and macrocirculation by increasing the saturation of damaged tissues with oxygen and improving their trophic supply by boosting metabolism and proliferation, thus initiating the development of recovery processes. These properties of LLLT make it an effective prevention and therapeutic tool that can be used to counter viral infection and its consequences and to prevent development of pulmonary fibrosis [37].

Laser therapy techniques

In the context of COVID-19 treatment, external laser therapy or intravenous laser blood illumination (ELD or IVLBT) are used

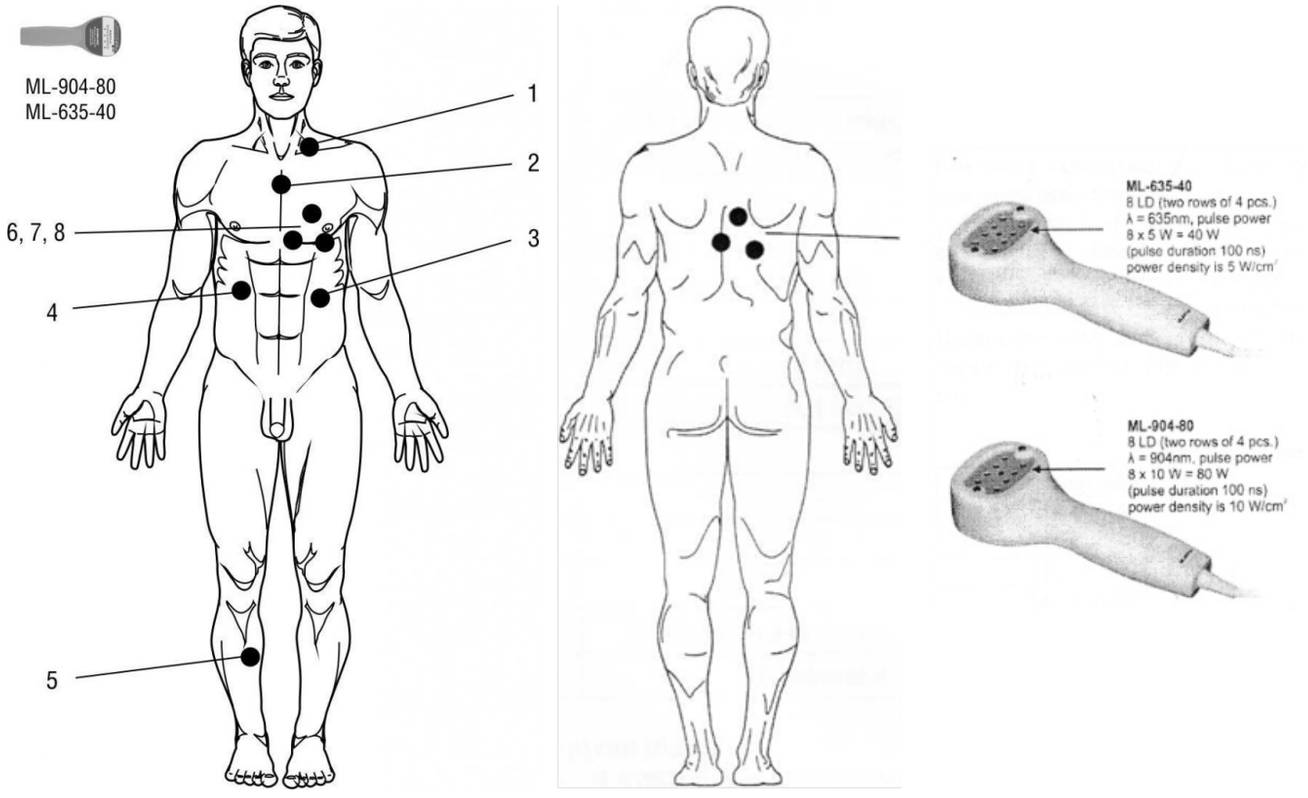

Fig. 2. Zones exposed to laser radiation from the emitting heads

Table 2. Zones exposed to laser light as a coronavirus disease prevention measure

Emitting head type	Exposed area (Figure 2)	Exposure, min
ML-635-40	1 — left supraclavicular region	2
ML-904-80	2 — thymus	1
ML-904-80	3 — spleen	1

Table 3. Parameters of the LLLT technique for prevention of coronavirus disease

Parameter	Value	Notes
Laser light wavelength, nm (spectrum)	635 (red)	–
	904 (IR)	
Laser operating mode	Pulse	Matrix emitting head, surface area 10 cm^2
Light pulse duration, ns	100–150	–
Radiation power, W	35–40	635 nm
	60–80	904 nm
Power density, W/cm^2	4–5	635 nm
	8–10	904 nm
Frequency, Hz	80	
Exposure per zone, min	See table 2	–
Number of exposed zones	3	–
Localization	See table 2	–
Method	Contact	Through the transparent tip
Number of procedures per regimen	2–3	Daily

to reach immunocompetent organs and applied locally, to the lesion focus [66]. This approach, a combination of exposure to LLLT on the systemic and local levels, has shown great results in clinical practice [66–69]. The most common technique used for the purpose of endothelium function correction is the "classical" wavelength of 635 nm, 2–3 mW output power and 10–20 min of exposure [70–76]. However, recently a combined version of the technique that includes laser UV blood illumination (LUVBI) has been gaining popularity [77–79]. The specialists are also well aware of the degrees of efficacy achievable in combinations of laser therapy and other physiotherapeutic methods, which have been confirmed in the treatment of COVID-19 [80–82].

LLLR-based coronavirus disease prevention and treatment

Those who have come into contact with the sick or who have arrived from epidemiologically unsafe areas are prescribed 2–3 LLLT procedures as prevention.

The sick receive treatment in hospitals; the regimen includes 10–12 daily laser therapy procedures.

Two versions of LLLT methods have been developed, the first relying solely on non-invasive techniques (external illumination) and the second, more effective, which involves IVLBT.

Method 1: prevention

Before starting the procedure, it is necessary to remove the protective cover and mount the magnetostatic field (MF) tip. The tip should be subjected to preliminary chemical sterilization (disinfection).

Fig. 2A and Fig. 2B show the zones (points) of application; Table 2 and Fig. 2C prescribe the type of emitting head and the exposure; Table 3 contains the parameters of the laser light.

Method 1: treatment

Before starting the procedure, it is necessary to remove the protective cover and mount the MF tip. The tip should be subjected to preliminary chemical sterilization (disinfection).

Fig. 2A and Fig. 2B show the zones (points) of application; Table 4 prescribes the type of emitting head and the exposure. Table 5 contains the parameters of the laser light.

Combined method 2

Combined method: external irradiation of zones 6-8 as shown on Fig. 2; type of emitting head and exposure as given in Table 4;

Table 4. Zones exposed to laser light in coronavirus patients

Emitting head type	Exposed zone (Fig. 1)	Exposure, min
ML-635-40	1 — left supraclavicular region	2
ML-904-80	2 — thymus	1
ML-904-80	3 — spleen	1
ML-904-80	4 — liver	2
ML-635-40	5 — E36 (zu san li) — symmetrical	0.5 min per zone
ML-904-80	6–8 — lung injury projection (see Fig. 2 for localization example)	1.5 min per zone

Table 5. Parameters of the LLLT technique for treatment of coronavirus patients

Parameter	Value	Notes
Laser light wavelength, nm (spectrum)	635 (red)	–
	904 (IR)	
Laser operating mode	Импульсный	Matrix emitting head, surface area 10 cm^2
Light pulse duration, ns	100–150	–
Radiation power, W	35–40	635 nm
	60–80	904 nm
Power density, W/cm^2	4–5	635 nm
	8–10	904 nm
Frequency, Hz	80	Zones 1–5
	80–1500	Zones 6–8, frequency can be varied depending on symptoms and patient condition
Exposure per zone, min	See table 4	–
Number of exposed zones	8	–
Localization	See table 4	–
Method	Contact	Through the transparent tip
Number of procedures per regimen	10–12	Daily

aser light parameters as provided in Table 5. Next step: IVLBT-525 + LUVBI (Table 6; Fig. 3).

Thirty-one SARS-CoV2-induced pneumonia patients with comorbidities (CVD, metabolic syndrome, type 2 diabetes mellitus, COPD, etc.) received rehabilitation treatment in the Central Clinical Hospital for the Rehabilitation of FMBA of Russia. In this group, the degree of damage to the lung tissue varied from 25 to 92%. Both of the above laser therapy methods were used for the patients; they delivered good results in the treatment of COVID-19 patients with severe lung lesions.

Subjectively, all patients noted general condition improvement, relief of chest pain associated with coughing, better sputum discharge, less severe shortness of breath. Moreover, in all patients we registered better oxygen saturation (pulse oximetry data) with the mean improvement from 93 to 97%; stabilization of the external respiration function accompanied by the increase of the vital volume of lungs; improvements revealed by the repeated lungs computed tomography. It is important that in the process of rehabilitation, these patients had their psychoemotional status normalized and the number of asthenic and anxiety-depressive incidents decreased (as measured with the Beck Depression Inventory and the MPS test (multilateral personality study).

The use of laser therapy for COVID-19 patients for the first time in the Central Clinical Hospital for the Rehabilitation of FMBA of Russia is mentioned as an example of the above promising non-drug therapies. As we gain experience, we shall report clinical data, more widely and in detail, with a statistical analysis of the results, evidence-based conclusions of the effectiveness of the method and personalized approaches in the complex treatment and prevention of complications.

CONCLUSION

This literature review demonstrates the capacities of laser therapy in the context of endothelial dysfunction treatment. The review cites positive experience of using laser therapy in the complex treatment and rehabilitation of patients with atypical pneumonia caused by various coronaviruses and the new SARS-CoV2.

LLLT is shown an absolutely safe, highly effective, simple and inexpensive method of prevention, treatment and rehabilitation of both chronic non-infectious cardiovascular and pulmonary pathologies and diseases caused by a viral infection, including COVID-19.

To enable personalized approach to rehabilitation of COVID-19 patients, it is necessary to search for informative biomarkers of genetic predisposition to endothelial dysfunction, hemostasis disorders, assess the individual characteristics of

Table 6. Parameters of the IVLBT 525 + LUVBI technique (basic)

Parameter	Value	Notes
Laser light wavelength, nm (spectrum)	365–405 (UV)	LUVBI
	520–525 (green)	IVLBT-525
Laser operating mode	Continuous	–
Radiation power *, mW	1,5–2	At the outlet of the disposable light guide
Exposure, min	3–5	LUVBI
	7–8	IVLBT-525
Localization	Vein ulnar median (v. mediana cubiti)	–
Method	Intravenously	Through the disposable sterile light guide KIVL-01 made by the Matrix R&D Center (TU 9444-005-72085060-2008)
Number of procedures per regimen	10–12	Daily, alternating every other day IVLBT-525 and LUVBI

innate immunity and adaptive immune response to infection, risks of hyperreaction, cytokine storm, multiple organ failure, delayed manifestation of complications in a particular patient. Determination of the contribution of these individual (hereditary and environmental) factors, consideration of their mutual influences are crucial for application of the results of such complex examinations in real practice and indispensable for the development of individual prognosis, prevention (primary and secondary) measures, targeted treatment regimens that, in particular, include LLLT.

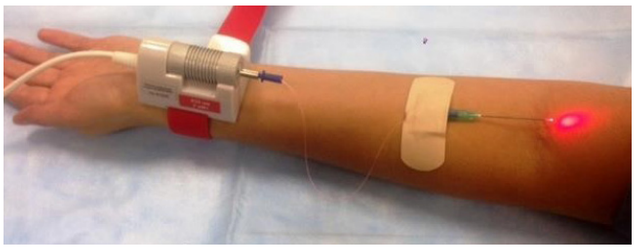

Fig. 3. IVLBT procedure

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SURGICAL CARE ARRANGEMENT AT THE GENERAL HOSPITAL DURING THE COVID-19 PANDEMIC

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The spread of caused by SARS-CoV2 acute respiratory infection associated with severe life-threatening complications has necessitated transformation of most general hospitals into infectious diseases hospitals in order to provide specialized care to infected patients, as well as the change of surgical care provision strategy. The example of surgical service reorganization has been reported for the general clinic transformed into the infectious diseases hospital capable of providing care both during the COVID-19 pandemic and after the outbreak has abated.

Keywords: COVID-19, SARS-CoV2, pandemic, coronavirus, surgery, surgical procedure

Author contribution: Nakatis YaA, Ratnikov VA, Kashchenko VA — study concept and design; Mitsinskaya AI, Mitsinskii MA, Akhmetov AD — data acquisition and processing; Lodygin AV, Mitsinskaya AI, Mitsinskii MA — manuscript writing; Kashchenko VA, Lodygin AV — manuscript editing.

Compliance with ethical standards: the patient submitted informed consent to treatment.

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ОРГАНИЗАЦИЯ ХИРУРГИЧЕСКОЙ ПОМОЩИ В МНОГОПРОФИЛЬНОМ СТАЦИОНАРЕ В УСЛОВИЯХ ПАНДЕМИИ COVID-19

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Повсеместное распространение острого респираторного заболевания, вызванного вирусом SARS-CoV2 и сопровождающегося развитием тяжелых, угрожающих жизни осложнений, привело к необходимости перепрофилирования большинства многопрофильных стационаров с целью обеспечения специализированной помощи инфекционным больным, а также потребовало изменения стратегии оказания хирургической помощи. Представлен пример реорганизации хирургической службы в исходно многопрофильной клинике, перепрофилированной в инфекционный стационар, способный оказывать помощь пациентам как в условиях пандемии COVID-19, так и после ее окончания.

Ключевые слова: COVID-19, SARS-CoV2, пандемия, коронавирус, хирургия, оперативное вмешательство

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Соблюдение этических стандартов: пациентка подписала добровольное информированное согласие на лечение.

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The COVID-19 (CoronaVirus Disease 2019) pandemic has been considered the largest outbreak of atypical viral pneumonia since 2002. In 2002 there was a similar, but less extensive, outbreak of SARS-CoV causing the severe acute respiratory syndrome (SARS) [1].

The World Health Organization (WHO) recognized the global spread of COVID-19 on March 11, 2020 [1]. The widespread ubiquitous infection with novel virus, lack of acquired immunity in the population, susceptibility among all age groups, as well as severe life-threatening complications made it necessary to introduce the measures to minimize the infection spread. These were self-isolation and quarantine together with transformation of most general hospitals into the infectious disease hospitals in order to provide specialized care to infected people [2]. The medical institutions modernization process went through a number of approbations of various tactics and schemes for transformation of non-infectious clinics into the infectious diseases hospitals.

The issue worth special attention is the arrangement of surgical care during the pandemic, since the high risk of viral contamination to operating team during surgical treatment of patients with COVID-19 without appropriate protection has been reported [3, 4]. It has now become evident that surgery during the pandemic requires taking into account a number of specific factors affecting surgical procedures both in patients with coronavirus infection and conditionally “clean” patients.

The general pandemic-related principles of surgical care arrangement are as follows. The surgical care of patients in the hospital should be limited to those whose needs are imminently life threatening. All elective surgical procedures should be postponed, and surgical priorities should be shifted to emergency care. The protocols of non-surgical management are currently being developed for patients whose surgical treatment may be postponed.

Transformation of the clinic into the infectious disease hospital for patients with COVID-19 results in certain matters impeding the work of surgical service. These include surgical beds elimination, surgical specialists’ redeployment, operating rooms used as intensive care units. The described issues may result in longer interval between diagnosis and surgery, reduced quality of surgical care and increased rates of complications [3]. Furthermore, the high risk of staff contamination during surgery calls for total revision of surgical care arrangement principles.

The key principle of safe and effective management includes constructing the clear hospital plan dividing the entire hospital area into "red" and "green" zones connected via single transition zone [2]. Operating room and intensive care unit should be located in the “red” zone.

Effective work is ensured by schedule optimization and mandatory presence of experienced surgeon in the on-duty surgical team. The surgeon should have time for consultations and surgical interventions.

To ensure safety and efficiency of surgical service during the pandemic the technical aspects of surgical intervention should be revised. Thus, surgery should be reduced to the minimum possible for current clinical situation extent. This will make it possible to reduce the duration of operation and to avoid the patient’s admission to the intensive care unit overloaded with severe COVID-19 patients. Moreover, electrocoagulation generates aerosol with high concentration of viral particles, which increases the risk of the operation room staff contamination. Consequently, the energy of electrocoagulation should be minimized. When technically possible, the use of electrocoagulation should be avoided.

The use of ultrasonic dissectors, monopolar electrosurgery and advanced bipolar devices should be minimized, since these can lead to the infected aerosol formation. It is better to use monopolar diathermy devices with attached smoke evacuators.

Laparoscopy requiring an artificial pneumoperitoneum is also an aerosol-generating procedure. The smoke leaking from abdominal cavity and produced by laparoscopic electrocoagulators has high concentration of viral particles, which necessitates the use of intelligent continuous-flow systems making it possible to maintain minimal intra-abdominal pressure and facilitating the smoke evacuation into a closed circuit [4].

Attention should be paid to the incisions length and port insertion method in order to prevent CO_2 leakage from abdominal cavity. Sudden removal of trocars should be avoided, and active aspiration should be used after the procedure. All CO_2 should be safely evacuated via a filtration system before closure [4]. Despite the proposed methods of laparoscopy techniques optimization in COVID-19 patients, it has been suggested that open surgery has some advantages in terms of operating room staff safety [5, 6]. There is no consensus on the presence of novel coronavirus in the peritoneum, but the presence of virus in the intestinal lumen and in the urinary tract is beyond doubt. This defines recommendations to consider the luminal opening or urinary drainage and bladder catheterization as additional risk factors for staff contamination. Thus, it is extremely important to prevent the infected aerosol formation and to minimize the operating room staff exposure to biological fluids.

The WHO issued a number of recommendations for surgical team management during the COVID-19 pandemic [4, 7]. The compliance with the recommendations for anesthesia is also important [8].

1. All manipulations to prepare the patient for anesthesia (central vein cannulation, endotracheal intubation) should be performed in the intensive care unit. After that the patient should be transferred to the operation room using the transport ventilator.

2. Sedative medications that may cause airway obstruction or hypoventilation requiring urgent intubation should be avoided.

3. The use of laryngeal masks, deep sedation and fiberoptic intubation in conscious patients should be limited.

4. If possible, regional anesthesia and the use of low-flow nasal cannula delivery systems should be preferred.

5. When performing surgery in COVID-19 patients, it is recommended to use low tidal volume ventilation with permissive hypercapnia and high positive end-expiratory pressure. In patients with refractory hypoxemia/hypercapnia or increased airway pressure, the use of prolonged neuromuscular blockade should be considered. The target SpO_2 (hemoglobin oxygen saturation) level is 88–92%.

During intubation and extubation of patients with novel coronavirus infection the following algorithm should be used [8].

1. The anesthesiology team which performs intubation should include two anesthesiologists or one anesthesiologist and the staff nurse wearing two pairs of gloves.

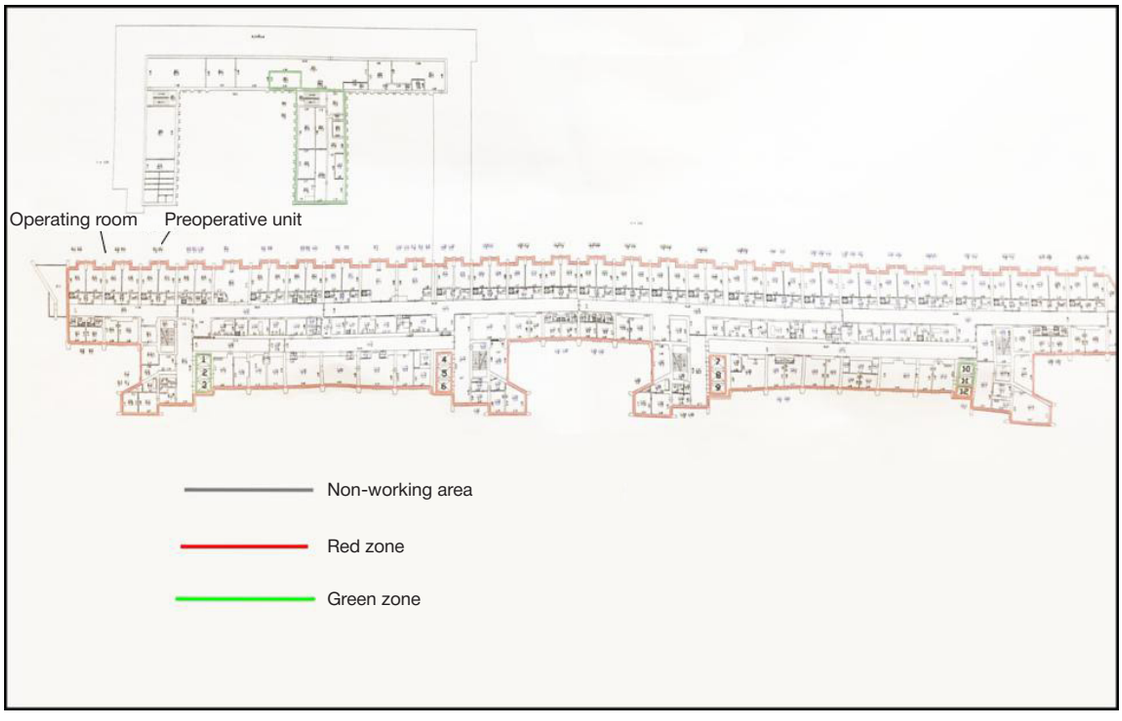

Fig. 1. Layout of operating room located in the red zone

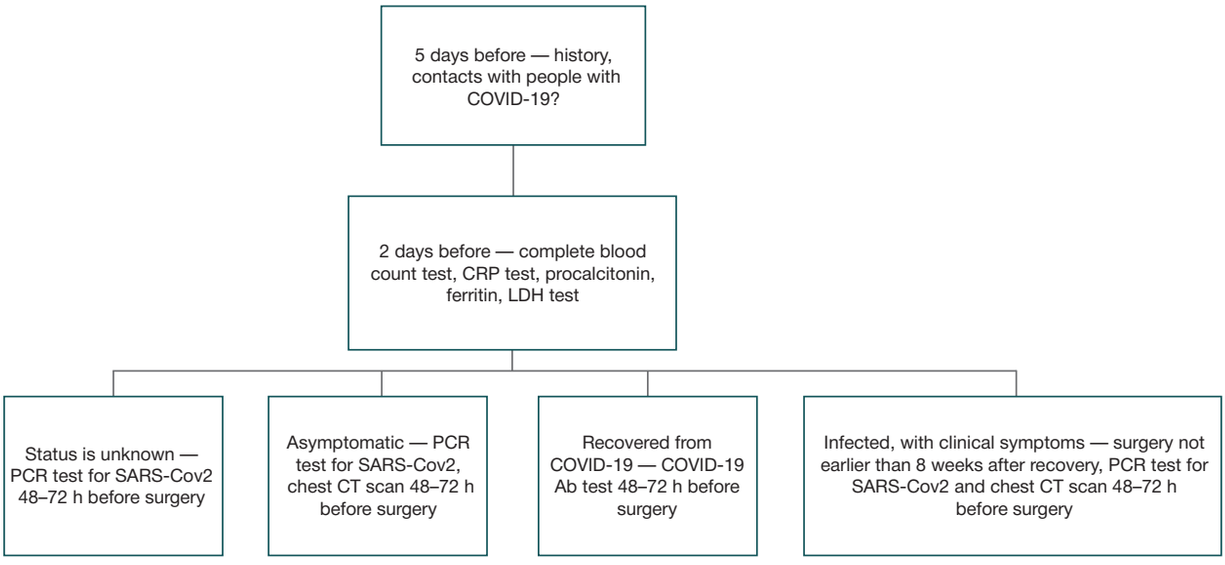

Fig. 2. COVID-19 screening pathway for patients in need for elective surgery. CRP — C-reactive protein; LDH — lactate dehydrogenase; PCR — polymerase chain reaction; chest CT — chest computed tomography; Ab — antybody

2. Extubation should be considered only in patients meeting criteria for extubation with no indications for intensive care unit stay.

3. During extubation only anesthesiologists and appropriate team members should stay in the operating room. The unnecessary staff should leave the operating room at the time of manipulation. They may enter the operation room again no earlier than 15 minutes after extubation completion.

4. The nurse wearing PPE should monitor the patient's awakening. Then the patient should be moved on the gurney and transferred to hospital ward using the surgical mask, gloves and protective disposable cap.

The patient's transfer to and out of the operating room should be as quick as possible. It should be performed away from other patients' accommodation. After that the thorough sanitization of all used facilities should be carried out.

Our aim was to report the surgical service arrangement at the L.G. Sokolov Memorial Hospital No. 122 transformed into the infectious disease hospital during the pandemic.

Clinical case

Prior to the COVID-19 pandemic a wide range of laparoscopic and open surgical procedures was performed at the L.G. Sokolov Memorial Hospital № 122. After transformation into the infectious diseases hospital all elective surgical cases were cancelled; only the life-saving surgical procedures were performed. The 350 hospital beds for infected patients were deployed. Fig. 1 provides the clinic floor plan showing the operating room suitable for emergency surgical patients with COVID-19.

The case of emergency surgery in patient with novel coronavirus infection and intra-peritoneal bleeding is reported.

The female patient B, aged 89, with clinical signs of bilateral community-acquired pneumonia and suspected COVID-19 was admitted to the L.G. Sokolov Memorial Hospital No. 122 on May 30, 2020. The diagnosis of COVID-19 was later confirmed by PCR test. Based on the clinical picture, patient's history, examination results, laboratory and instrumental tests, the following diagnosis was established:

Primary diagnosis: coronavirus disease caused by SARS-CoV-2, virus identified, severe course.

Complications: community-acquired bilateral polysegmental pneumonia (CT-3). III degree respiratory failure.

Secondary diagnosis: coronary artery disease. III class stable angina pectoris. Atherosclerotic cardiosclerosis. Grade 3 essential hypertension. Grade 3 hypertension. Very high risk of cardiovascular complications.

Due to severity of the disease, the patient was hospitalized in the cardiac intensive care unit. On June 1, 2020 the following hemodynamic changes were observed: blood pressure drop to 80/60 mmHg, tachycardia 140 beats per minute, and the need for sympathomimetic therapy. The complete blood count (CBC) test results showed the pronounced decline in hemoglobin level over time to 45 g/L (severe anemia). Abdominal CT scan revealed signs of spleen rupture and hemoperitoneum (it was also known from the case history that the patient fell off in her apartment on May 29, 2020). The patient was in need of live-saving surgery.

After intubation, performed in the intensive care unit by the equipped with PPE anesthesiology team members, the patient was prepared for urgent surgery. The surgical team was provided with PPE and P100 (HEPA) full-face respiratory protection equipment. A sterile surgical gown and sterile latex gloves were worn over the PPE.

Laparotomy using the monopolar electrocoagulator with lowest possible power setting was performed. Abdominal cavity revision revealed 2000 mL of blood with clots. Vizualization of the spleen revealed a linear rupture near the upper splenic edge. Splenectomy, peritoneal debridement and drainage were carried out. After surgery the patient was transferred to the intensive care unit for further treatment without extibation. No surgical complications were detected during the postoperative period. During the next week, the underlying disease progression was noted. Despite the intensive conservative therapy, the patient died on the day 10 of hospital stay due to comorbidities and age factor, as well as to progressive respiratory failure.

The control nasopharyngeal swab samples tested by PCR were SARS-Cov2 negative in all team members. Monitoring of the operating room staff over the next 14 days also revealed no clinical signs of COVID-19.

Discussion

The clinical case reported proves the need to elaborate principles for the correct surgical care provision to patients

with novel coronavirus infection. Consistency throughout the recommendations makes it possible to ensure safety of patient and staff during surgery. Moreover, the COVID-19 spread not only contributes to the need for surgical tactics correction during the pandemic, but also defines the further surgical service reorganization strategy after the outbreak has abated.

In case of epidemiological situation stabilization and incidence plateau it is necessary to gradually expand the range of surgical services provided. In case of no disease outbreaks and minimized person-to-person transmission the elective surgery may be resumed.

The activities should be resumed after screening of all healthcare workers for COVID-19. Next step is the institutional resources evaluation.

The decision about elective surgery should be made based on the surgical care promptness, institutional resources availability (including the intensive care unit bed availability), disease severity, history of cancelled surgical procedures and the patient's demographic data. In order to minimize the spread of infection, all patients with suspected COVID-19 requiring surgical treatment should be considered positive until proven otherwise [9]. Pre-operative assessment should be performed in accordance with the following protocol [10] (Fig. 2).

Maximum reduction of rehabilitation period contributes to reduced hospital length of stay. Referrals to rehabilitation centers should be avoided; on day 12–14 after discharge another nasopharyngeal swab tested for SARS-Cov2 by PCR should be performed [11].

It is important to remember that prevention of nosocomial COVID-19 outbreaks requires respect for discipline at work, patient routing and strict adherence to principles of asepsis and antisepsis.

CONCLUSION

Current epidemiological situation that has developed due to the COVID-19 spread contributes to the change of surgical care provision strategy in the clinics transformed into infectious diseases hospitals, and makes it necessary to revise the surgical care principles after the pandemic has abated. The change affects both surgery in patients with coronavirus infection and surgical treatment in conditionally "clean" patients during a period of unfavorable epidemiological situation. Selective and standardized approach together with strict adherence to recommendations ensures high efficiency of surgical care provision and safety of healthcare specialists.

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