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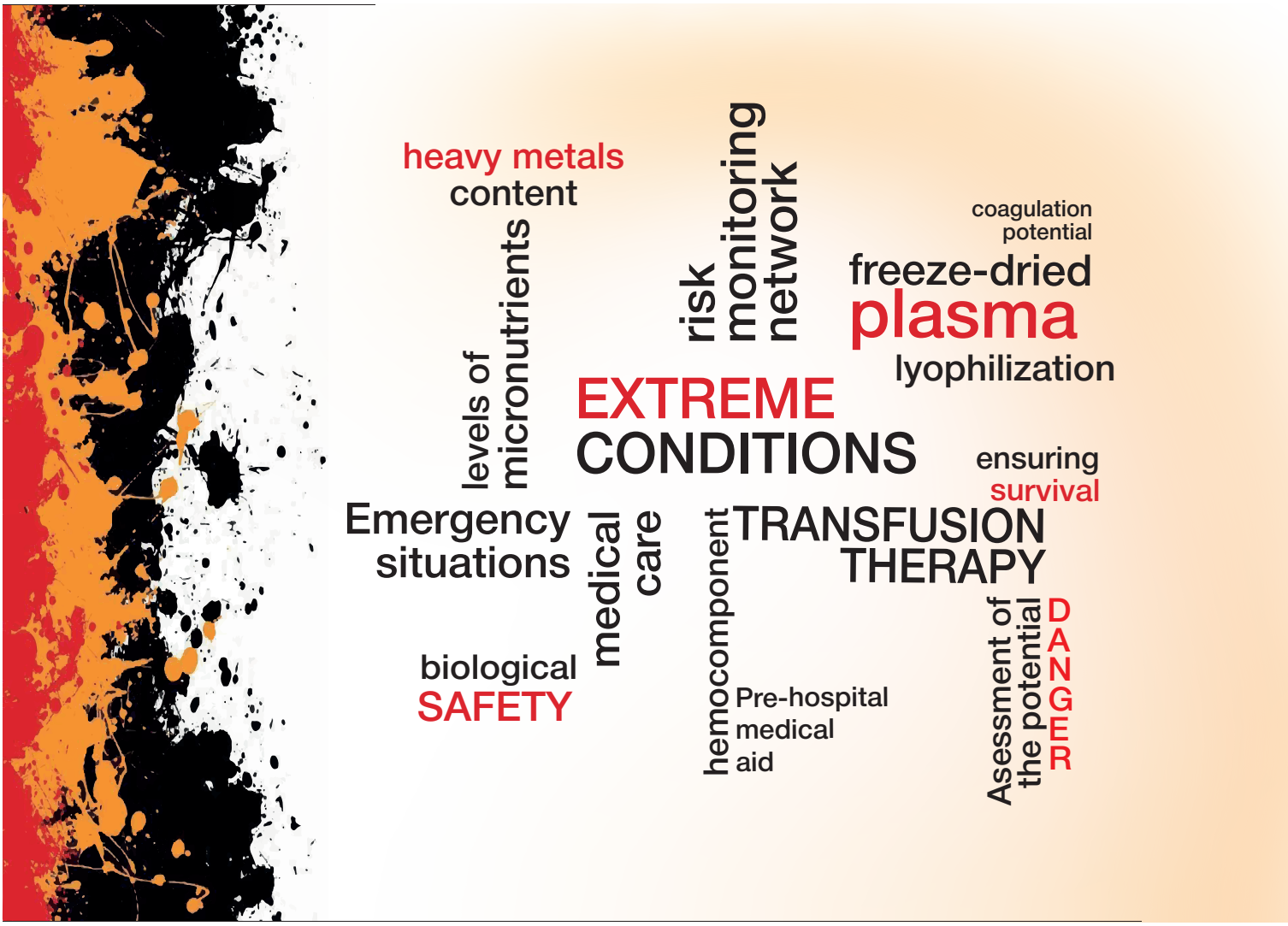

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FREEZE-DRIED PLASMA FOR EMERGENCY TRANSFUSION CARE IN EXTREME CONDITIONS

Svetlana E. Ziganshina[1]✉, Elena S. Kormshchikova[1], Elena N. Kalinina[1], Elena V. Rosina[1], Ekaterina A. Konovalova[1], Sergey V. Ignatyev[1], Aleksey V. Lyanguzov[1], Olga V. Eihler[2], Konstantin A. Vorobiev[1], Igor V. Paramonov[1]

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Introduction. In the context of limited availability of fresh frozen plasma, the use of freeze-dried plasma offers significant logistical advantages in extreme conditions. The effectiveness of freeze-dried plasma depends on the preservation of coagulation potential in the manufacturing process.
Objective. Review of research achievements both in Russia and aboard in the field of freeze-dried plasma technologies, including manufacturing, quality control, and blood component application.
Discussion. Commercial products such as FLyP, LyoPlas N-w, Bioplasma FDP, OctaplasLG Lyo, as well as freeze-dried plasma (Belarus or China), which have proven their effectiveness and safety, are available in glass vials. The production of freeze-dried plasma in polymer containers using membrane technology is a promising direction offering the advantage of using blood components in extreme conditions. The freeze-dried plasma products developed by Terumo BCT Biotechnologies and Teleflex Inc. are currently undergoing clinical trials and are used in military operations to a limited extent. In the Russian Federation, the Lyokon polymer container has been registered. During the lyophilization process, the pH increases to alkaline pH values of 8, which is associated with the removal of carbon dioxide. When assessing the coagulation potential, the most significant decrease is observed in the activity of factor VIII — up to 50%, factor V — up to 37%, protein S — up to 34%, and von Willebrand Factor — up to 25%. The prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT) is noted. In the Russian Federation, freeze-dried plasma belongs to the group of blood components; therefore, the introduction of foreign production experience (the introduction of cryo- and lyoprotectors, pH adjustment, etc.) is restrained by legislation. This emphasizes the importance of developing domestic technologies.
Conclusions. The production of freeze-dried plasma in polymer containers contributes to uninterrupted transfusion support in the provision of medical care, thus increasing the survival rate of the injured with acute blood loss in emergency situations. In this regard, creation of domestic plasma lyophilization technologies and enhancement of their effectiveness are relevant tasks.

Keywords: freeze-dried plasma; production technology; lyophilization; coagulation potential; transfusion therapy

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ЛИОФИЛИЗИРОВАННАЯ ПЛАЗМА ДЛЯ ОКАЗАНИЯ ЭКСТРЕННОЙ ТРАНСФУЗИОЛОГИЧЕСКОЙ ПОМОЩИ В ЭКСТРЕМАЛЬНЫХ УСЛОВИЯХ

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Введение. В условиях ограниченных возможностей применения свежезамороженной плазмы в экстремальных условиях важны логистические преимущества, которые дает использование лиофилизированной плазмы. Эффективность ее применения зависит от сохранности коагуляционного потенциала в процессе производства.
Цель. Анализ перспективных направлений совершенствования технологий получения лиофилизированной плазмы с использованием международного и отечественного опыта производства, оценки контроля качества и применения гемокомпонента.
Обсуждение. Применяющиеся и доказавшие свою эффективность и безопасность коммерческие препараты FLyP, LyoPlas N-w и Bioplasma FDP, OctaplasLG Lyo, а также лиофилизированная плазма Республики Беларусь и КНР выпускаются в стеклянных флаконах. Перспективным направлением считается получение лиофилизированной плазмы в полимерных контейнерах с применением мембранной технологии, что обеспечивает преимущества использования гемокомпонента в экстремальных условиях. Известны разработки компаний Terumo BCT Biotechnologies и Teleflex Inc., полученные ими продукты лиофилизированной плазмы находятся на стадии клинических исследований и ограниченно применяются в военных операциях. В Российской Федерации зарегистрирован полимерный контейнер «Лиокон». В процессе лиофилизации наблюдается увеличение pH до щелочных значений порядка 8, что связано с удалением углекислого газа. При оценке коагуляционного потенциала наиболее значимо снижение активности фактора VIII до 50%, фактора V — до 37%, протеина S — до 34%, фактора Виллебранда — до 25%. Отмечена пролонгация протромбинового

времени (ПВ) и активированного частичного тромбопластинового времени (АЧТВ). В Российской Федерации лиофилизированная плазма относится к гемокомпонентам, поэтому внедрение зарубежного опыта производства (внесение крио- и лиопротекторов, корректировка pH и др.) ограничено законодательно, что подчеркивает важность разработки отечественных технологий.
Выводы. Производство лиофилизированной плазмы в полимерных контейнерах является одним из путей бесперебойного трансфузионного обеспечения при оказании медицинской помощи, что будет способствовать повышению выживаемости раненых с острой кровопотерей в чрезвычайных ситуациях. В связи с этим актуально создание отечественных технологий лиофилизации плазмы и разработка подходов к повышению ее эффективности.

Ключевые слова: лиофилизированная плазма; технология получения; лиофилизация; коагуляционный потенциал; трансфузионная терапия

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INTRODUCTION

The availability of blood component therapy is extremely important for patients with massive blood loss already at the stage of their medical evacuation [1, 2]. In treatment of post-traumatic coagulopathy, the use of donor plasma as a source of physiological procoagulants and anticoagulants, activators and inhibitors of fibrinolysis is justified [3, 4, 5]. The use of fresh frozen plasma (FFP) in extreme conditions, including remote and hard-to-reach areas, at sea, and during air transportation, is complicated by logistical factors and the impossibility of providing a cold chain. The limited use of FFP is also associated with the fragility of containers with frozen blood components and the high risk of damage during transfer with plasma transportation and thawing. Up to 40% of containers are written off as expenditure due to defects [6]. Prior to transfusion, the blood component must be thawed and warmed, which requires time and specialized equipment. Similar problems in the provision of transfusion care arise in cases of mass destruction due to natural or man-made disasters accompanied by damage to infrastructures [4–8].

Due to logistical challenges of using FFP in extreme conditions, dry plasma offers significant advantages, including the ease of transportation and preparation for transfusion, as well as its long shelf life. This increases the availability and efficiency of transfusion care in life-threatening conditions [4–8].

Lyophilization is an effective method of dry plasma production [9]. Liquid plasma is subjected to shock freezing following its freeze-drying under low vacuum conditions (less than 35 Pa). The solvent is removed from the product by its transfer from the frozen state to the gaseous state during gradual heating in the temperature range from –45 °C to +35 °C, which reduces the loss of functional activity of the target proteins during dehydration.

Freeze-dried plasma (FDP) is included in the list of blood components approved by Enactment of the Government of the Russian Federation No. 797 (dated 22.06.2019)[1].

Another method for biomaterial dehydration is spray drying, when plasma is dispersed in a stream of hot air at a temperature of 60–150 °C [5, 7, 10, 11]. In comparison with freeze-drying technology, spray drying requires no sophisticated equipment but ensures high productivity. However, the as-dehydrated plasma is not available in European countries; in the USA, such technologies are currently under development and clinical trials [10, 11]. In Russia, spray-dried plasma is not included in the list of blood components[2].

The effectiveness of FDP largely depends on the preservation of blood coagulation factors, natural anticoagulants, and other proteins that enable plasma hemostasis [3–5]. In Russia, FDP can be obtained from quarantined or pathogen-reduced plasma[3]. The technological process includes shock freezing, thawing and refreezing, exposure to light and chemical agents to inactivate pathogens, as well as direct freeze-drying. These stages have a significant impact on the structure and functional activity of plasma proteins, especially thermolabile ones, which include blood clotting factors [4, 5, 7, 12]. In this regard, the technological parameters of FDP production should be selected such that to ensure its maximum preserved coagulation potential and safety parameters in full compliance with regulatory requirements.

In this article, we review promising directions in the field of freeze-dried plasma production technologies,

[1] Enactment of the Government of the Russian Federation No. 797 (dated 22.06.2019) “On approval of the Rules for Procurement, Storage, Transportation and Clinical Use of Donated Blood and Its components and on the Invalidation of Certain Acts of the Government of the Russian Federation.” Moscow: RF Government; 2019.
[2] ibid.
[3] ibid.

both in Russia and abroad, evaluating approaches to quality control and use of the blood component.

MATERIALS AND METHODS

The literature search was conducted across electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (PubMed, Web of Science, Scopus) languages, as well as patent sources (Google Patent Search, FIPS). The search queries included the following keywords: lyophilized plasma, freeze-dried plasma, production technology, lyophilization, coagulation potential, transfusion therapy (lyophilized plasma, technology of acquiring, lyophilization, coagulation potential, transfusion therapy). The search depth was 10 years. The publications containing information on freeze-dried plasma production technologies and promising developments in this field were included in the review.

RESULTS AND DISCUSSION

First experiments on the production and application of freeze-dried plasma

The FDP production technology was developed in the 1930s [3, 5, 7, 8, 13, 14]. In 1939, specialists of the Leningrad Institute of Blood Transfusion (since 2011, Russian Research Institute of Hematology and Transfusiology) under the supervision of Prof. L.G. Bogomolova developed one of the world's first chamber-type sublimation devices, which marked an important stage in the development of lyophilization in Russia [14]. Research efforts in the field of blood plasma lyophilization and the development of respective equipment were undertaken in the UK, USA, Canada, and other countries [3, 5, 7, 8, 13].

Large-scale FDP production began during World War II. Millions of dry plasma units were supplied from the USA and Great Britain to the allied forces [5–8]. In the USSR, even during the blockade, FDP production was carried out in Leningrad (1941–1944), primarily for the needs of the Baltic Fleet. Blood components were produced mainly from blood plasma of the AB (IV) blood group, packaged in glass bottles or ampoules [14, 15].

In the USSR, FDP had been produced on an industrial scale since the 1960s. Plasma was manufactured according to the following standard regulations. Prefreezing was carried out in glass vials in alcohol baths. To distribute the plasma over the surface of the vial, the containers were rotated at an angle of 3–5° around the horizontal axis. This process yielded the thinnest possible layer of frozen product and allowed the evaporation surface to be extended. This approach, as well as the selected lyophilization regime, made it possible to dehydrate the plasma to a residual humidity of less than 1% within 20–26 h or 28–32 h, depending on the lyophilization device used. All technological operations were carried out in compliance with aseptic technique and sterility control. For better preservation of plasma proteins, a glucose-based protective medium was used. During lyophilization, a sterile solution of this monosaccharide (5 or 40%) was added to plasma in a 1:9 ratio [9, 16].

The finished products were monitored according to the following quality parameters: solubility — no more than 10 min, authenticity — formation of a dense clot in the presence of a 5% calcium chloride solution (qualitative reaction to the presence of fibrinogen), residual moisture — less than 1%, sterility — sterile, total protein — not less than 55 g/L. The shelf life of such a medicinal product was 5 years at a storage temperature of 5–25 °C. According to the results of studying the product stability after 8 years of storage, the dissolution time increased 2.5 times without exceeding the norm (4–10 min); the remaining parameters did not change significantly. The pH of the FDP was close to neutral, comprising 7.5 ± 0.2 [9]. However, it should be noted that in the 1960s, it was not possible to assess the hemostasis system parameters, their level in production batches was not normalized, and the shelf life was set without taking into account the dynamics of the activity of thermolabile proteins during storage.

FDP was used mainly in cases where it was not possible to transfuse donated blood; its use proved its effectiveness in the wounded with acute blood loss and traumatic shock. The large-scale production of FDP was discontinued in the 1980s due to the detection of cases of hemotransmissive viral infections (HTVI). Attempts made to reduce the infection risk of recipients by removing viruses and reducing the plasma pool size were ineffective [3, 5–8].

Development of freeze-dried plasma production technologies

Freeze-dried plasma technologies have received a new impetus for development since the emergence of reliable standardized methods for ensuring virus security. In 1991, to meet the need for blood transfusions and its components during military operations in the Persian Gulf, FDP production was resumed in France [5–8]. Safety was ensured by the formation of small plasma pools (less than 11 donors) with strict control over the absence of HTVI markers, plasma quarantine and repeated examination of donors. An additional measure to increase the product safety was the introduction of blood plasma screening from females for the presence of antibodies to the human leukocyte antigen.

The French Military Blood Institute produces FDP under the trade name of FlyP. It is a pooled, pathogen-reduced by amotosalene and ultraviolet radiation, AB0-universal FDP. In order to ensure the required level of factor VIII in the dry blood component, taking into account the effect of pathogen reduction, FFP with an activity of at least 0.96 IU/mL is preferred [17]. After thawing, the

selected plasma doses are pooled, aseptically poured into glass vials, and freeze-dried. The lyophilization process lasts for 4–6 days [17–20].

In the early 1990s, the Blood Service of the German Red Cross began producing pulled FDP (up to 1000 donors) treated with a solvent-detergent method. Due to the concerns that the technology used was not capable of inactivating the prions that cause Creutzfeldt-Jakob disease, since 2007, the pooled plasma has been replaced with a single donor FDP. Currently, LyoPlas N-w is a single-donor quarantined FDP. The plasma is stored frozen for at least four months until the donor is examined again, then it is thawed and connected to a patented sterile filling system consisting of a glass vial and a rubber stopper inside a plastic bag [5–8, 20]. Plasma (200 mL) is poured into the vial through a filter with a nominal pore size of 0.2 μ; the vial is closed with a stopper and removed from the system. The plasma is then frozen to –30 °C. Drying occurs with a stepwise increase in temperature from –45 °C to +15 °C for six days; the residual humidity of the plasma is no more than 1% [5, 20].

The commercial Bioplasma FDP product has been manufactured by the National Bioproducts Institute of South Africa since 1996. It is pulled, treated with a solvent-detergent method, AB0-universal FDP [6–8, 20].

Since 2016, the Republican Scientific and Practical Center for Transfusiology and Medical Biotechnology of the Republic of Belarus has been actively developing pooled (at least 10 units of plasma), pathogen-reduced (photochemical treatment using riboflavin or amotosalene), fibrinogen-standardized FDP. To reduce the loss of blood clotting factors during lyophilic drying, auxiliary substances are added to the intermediate product, the composition of which is not disclosed [21, 22].

The Swiss company Octapharma AG has received approval from European regulatory authorities for the production of OctaplasLG Lyo FDP [23]. It is obtained from a pool consisting of 630–1520 units of single-group donor plasma, which is filtered to remove aggregates and cell fragments. A solvent-detergent treatment method is used to ensure virus safety. The fundamental difference between this technology and the previously described ones is the stage of chromatographic purification using affinity ligands for prion proteins. The plasma is subjected to sterilizing filtration and bottled in non-pyrogenic glass vials of 200–210 mL followed by lyophilization. It should be noted that the production stages of OctaplasLG Lyo are accompanied by pH adjustment using citric acid or phosphoric acid to compensate for the increase in this indicator during the lyophilization process. Glycine in a final concentration of 5 g/L is used as a stabilizer. Prior to application, the FDP is rehydrated in 190 mL of water for injection [20, 24].

Plasma lyophilization technologies, including platelet-rich plasma, have been patented in China (Institute of Pharmacology and Toxicology of AMMS, First Medical Center of PLA General Hospital, Qilu Cell Therapy Technology Co ltd Yinfeng Biological Group Ltd). The blood component is dried for 4–6 days. The material is cooled to –45 °C, then gradually heated to +20 °C at a vacuum value of 0.1 mbar. At the secondary drying stage, the pressure is reduced to 0.001 mbar, and the temperature is raised to +25 °C [25–27].

The above FDP products are produced in glass vials. The disadvantages of this package are fragility, bulkiness, and significant weight. Care is required during transportation, which is quite difficult to ensure in extreme conditions. In addition, for the filling stage and lyophilization itself, it is necessary to create aseptic conditions to prevent contamination of the product [28–29].

Innovative technologies for producing freeze-dried plasma in polymer containers

The inconveniences of transporting and using FDP in glass vials drive the need to develop technologies for producing freeze-dried plasma in polymer containers. The lightness, compactness, strength, and tightness of such consumable systems increases the availability and efficiency of early transfusion therapy in extreme situations outside of inpatient conditions. To date, the most promising direction has been the membrane lyophilization technology, when one of the container surfaces is made of a gas-permeable polymer. This material is highly hydrophobic and non-toxic, capable of preventing the penetration of microorganisms and at the same time being permeable to water vapors. All this allows the FDP manufacture in a closed system while maintaining the sterility contour, thus offering the advantages of using dry blood components in extreme conditions [8, 28, 29].

Abroad, the United States is the leader in the development of FDP production technologies in polymer containers. In 2007–2008, the U.S. Army Medical Materiel Development Activity (USAMMDA) and the U.S. Army Special Operations Command (USASOC) launched programs for the production of FDP in polymer containers. In 2008–2013, HemCon Medical Technologies, Inc. was a partner of the U.S. Department of Defense. Although plasma pooling ensured its standardization in terms of the level of blood clotting factors, preference was given to FDP obtained from a single donor. In 2011, this product successfully passed the first phase of clinical trials. Afterwards, however, the collaboration with HemCon Medical Technologies, Inc. was terminated. In 2014, together with a new partner Vascular Solutions, Inc., the FDP under the commercial name of RePlas was developed and passed the first phase of clinical trials [5, 7, 20, 30]. This company also produces ESPLAS, an FDP from a single donor [8].

In 2016, Terumo BCT Biotechnologies, LLC, a biotech company, received funding to develop a decentralized FDP production in polymer containers from plasma pools (up to 10 donors) for use in blood centers and extreme situations. Currently, the technology

and consumables developed by this company for FDP production are used not only in the USA, but also in Canada [31–33].

The gas permeable membrane of lyophilization polymer containers developed in the USA is made on the basis of foamed polytetrafluoroethylene (e-PTFE). The choice of this material is due to its porous and flexible structure, chemical stability, and biocompatibility [34]. The presence of negative charges on the polymer surface blocks the coagulation of blood proteins and limits platelet activation. The pore size of the lyophilization container membrane ranges within 0.2–0.3 μ, ensuring protection of the product from microbial contamination. The porosity of 50–95% allows efficient removal of liquid vapors. The developed containers are presented in a two-piece design. One of the parts is equipped with a gas-permeable membrane, while the other is made of a non-breathable polymer material such as polyvinyl chloride or polypropylene. To increase the sublimation efficiency during the process, the plasma does not come into contact with the membrane surface [35–37].

The design of Terumo BCT Biotechnologies, LLC containers may include a temporary seal in the form of a reinforcing insert separating a part of the plasma container and an unfilled section with a breathable membrane. In this case, an occlusion area is required to create an air space in order to accelerate the outflow of solvent vapors during the sublimation process [35, 36]. In the design of Teleflex Inc. containers, a device can be provided in the form of a frame made of inert medical plastic supporting the membrane above the plasma layer [37].

The duration of plasma drying in such polymer containers is comparable to that of lyophilization in vials, being about 4–7 days. After lyophilization, the dry product is stored in the non-breathable part of the container (or poured thereon, if necessary), which is separated from the membrane section by a sealed seam. The container in which the FDP is stored is equipped with the ports for solvent injection and transfusion of rehydrated plasma [35–37].

A number of developments in the field of plasma lyophilization in polymer containers are also known in the Russian Federation. In 2021, Haemogenics patented a system consisting of a container comprising two sections hermetically connected by a peel-open heat-sealed seam, which makes it possible to freeze, store and use the blood component while maintaining the sterile contour [38]. A non-woven polymer Tyvek is used as an air-permeable material, which performs the function of a membrane. Plasma drying is carried out in the gas-permeable part of the container. The second section, into which the lyophilizate is poured at the end of the process, is made of polyvinyl chloride and is used for storage, transportation, and transfusion of the blood component. A similar principle was implemented by NPO Biotech-M when developing a method for plasma lyophilization in a two-section container, characterized by the design features of the intersectional seam, a different composition of the breathable material, and the configuration of ports and tubing lines [39]. Later, the authors noted that the main disadvantage of binary containers is their large area, which requires a significant increase in the working surface of the freeze chamber. The membrane materials are hygroscopic; therefore, the transfer of FDP from one section to another can lead to a significant increase in the moisture content of the blood component [40].

Single-section containers are easy to manufacture and are free from the above disadvantages. Currently, such containers are registered as a Liokon medical device (NPO Biotech-M, Russia) and are used for plasma drying according to a protocol integrated into the software of the Liomed lyophilization unit (the same producer). The containers are made in the form of a flattened container with an area of about 420 cm^2 (linear size 15.5 cm×27.3 cm). One of its surfaces is made of water-, gas-, and vapor-proof material, the other is a membrane with a pore size in the range of 0.1–0.45 μ and a porosity of 20–80%. Lyophilic drying of plasma in these containers is carried out at temperatures from –40 to +37 °C for 4–7 days. After completion of lyophilization, immediate sealing of the membrane surface is necessary. For additional protection against ingress of moisture from the environment and damage during storage and transportation, the container is placed in an external bag and evacuated [41].

Research is underway in the institutions of the Federal Medical and Biological Agency (FMBA of Russia) to develop production technologies of FDP in polymer containers [42]. Since 2024, the Kirov Scientific Research Institute of Hematology and Blood Transfusion has been working on the production of dry plasma with increased coagulation potential using membrane technology as part of a versatile package to provide emergency transfusion care to the wounded and injured with massive blood loss in extreme situations.

Use of pathogen reduction technologies to ensure the infectious safety of freeze-dried plasma

Pathogen reduction technologies make it possible to increase the infectious safety of blood components. Such technologies are aimed at removing a wide range of viruses, and not just four HTVI, the detection of which is mandatory during a medical examination of donors [5, 43]. There exists evidence that modern technologies prevent hemotransmissive bacterial sepsis. The introduction of the pathogen reduction stage eliminates a long period of quarantine, avoids the rejection of the product due to the failure of donor re-examination, and reduces the time to obtain a suitable blood component for clinical use [43].

From the point of view of ensuring viral safety, the single-donor FDP production is preferable. However, the wide variability of the plasma physiological

parameters makes it difficult to ensure the quality of the finished product. Combining plasma units into a pool makes it possible to standardize the product in terms of total protein, blood clotting factors, fibrinogen, and natural anticoagulants. In this case, the introduction of the pathogen reduction stage is of particular importance [7, 28].

The solvent-detergent method, introduced in 1991 as an alternative to quarantine, is used to process plasma pools (up to hundreds and thousands of units). The disadvantage of this method consists in a decrease in the activity of natural anticoagulants: protein S up to 44% and α_2-antiplasmin up to 79% [44].

Methods based on photoinactivation of pathogens are used to process individual plasma units and pools of up to 2–3 units. Such technologies involve visible light and methylene blue treatment (THERAFLEX system, Macopharma, France) for plasma doses of 235–315 mL, ultraviolet irradiation with riboflavin (Mirasol system, Terumo BCT, USA) for plasma doses of 170–360 mL, ultraviolet in combination with amotosalene (INTERCEPT system, Cerus Corporation, USA) for apheresis doses of plasma with a volume of no more than 650 mL or pooled plasma with a volume of 385–650 mL obtained from whole blood [43].

The conducted comparison of photochemical technologies for pathogen reduction [43] found their effect on the plasma coagulation potential. In 2014, Jose Coene et al. noted a decrease in fibrinogen concentration (16.8–33.2%), activity of factors II (2.2–22.6%), V (7.8–38.2%), VIII (22.3–44.7%), IX (9.2–33.9%), and XI (14.8–47.4%). The greatest change in the plasma hemostatic properties was observed when irradiating plasma with ultraviolet light and treating with riboflavin [45]. The same trend was observed when studying the coagulation potential of pathogen-reduced FDP produced in Belarus using the Mirasol and INTERCEPT systems. The decrease in the activity of factor VIII was 39.3% and 19%, and the decrease in fibrinogen content was 33.6% and 25.3%, respectively [21]. The [46] of plasma drying technologies in 10 mL glass vials involving three pathogen reduction methods found no significant effect of the viral activation method of the biomaterial on the preservation of its hemostatic properties. The work observed a decrease in the activity of factors V and VIII by 18–20% and 15–19%, respectively, as well as an increase in PT and aPTT compared with the same parameters in the FDP. The remaining parameters ranged within the physiological norm [46].

The introduction of pathogen reduction technologies requires specialized equipment and expensive consumables. At the same time, the expenditures are justified by increasing safety, reducing the duration of the FDP production, and making more rational use of the donor resource [43, 46].

Production of group AB(IV) freeze-dried plasma

FDP can be effectively applied only provided the comparability of the AB0 system between donor and recipient. The use of group AB(IV) plasma in extreme conditions provides a time advantage and reduces the risk of transfusion of a blood component incompatible with the blood group. Therefore, due to the possibility of immediate transfusion, products based on the group AB(IV) blood component are in high demand [6, 28, 29].

According to literature data, the AB(IV) blood group prevalence among the population is only 8–9%. To increase the availability of plasma transfusions in extreme situations, it is allowed to use plasma with a low titer of anti-A antibodies as a “universal” plasma or to combine plasma of groups A, B, and AB in certain proportions. For example, there is a known method for forming a pool for FDP production with a relative content of individual plasma units of group A(II): 40–45%, group B(III): 40–45%, group AB(IV): 10–20% [6, 17, 19, 28, 29].

In accordance with Enactment of the Government of the Russian Federation No. 797[4] (dated 22.06.2019), in the absence of same-group plasma, only AB(IV) plasma transfusion is allowed. In this regard, the formation of a reserve of AB(IV) donor plasma is of strategic importance for Russian healthcare.

Studying the properties of freeze-dried plasma

During plasma lyophilization, it is important to maximize its coagulation potential, i.e., the activity of blood clotting factors and natural anticoagulants, and the concentration of fibrinogen. At the same time, these values should be considered in conjunction with the data of global coagulological tests, such as thromboelastography. Humidity is controlled in the finished product. Under its of less than 2%, it is believed that FDP stability is ensured during long-term storage. The amount of total protein is determined, and a sterility test is performed. In addition to the main quality parameters, the physicochemical properties and FDP composition are verified by the dissolution time, pH, osmolarity, and residual concentrations of excipients.

When studying the properties of FLyP, the following values of coagulation potential were obtained: fibrinogen level — 2.4 ± 0.3 g/L; factor V activity — 0.51 ± 0.16 IU/mL; factor VIII — 0.62 ± 0.10 IU/mL; factor IX — 0.79 ± 0.11 IU/mL; factor XIII — 1.03 ± 0.12 IU/mL; protein C — 96 ± 9%; protein S — 77 ± 16%; antithrombin III — 1.01 ± 0.05%; α_2-antiplasmin — 95 ± 30%. At the same time, out of the nine studied parameters, only two showed a significant decrease during lyophilization. The activity of factors V and VIII decreased by 25 ± 12 and 20 ± 7%, respectively. The remaining parameters were stable, varying within 7%.

[4] Enactment of the Government of the Russian Federation No. 797 (dated 22.06.2019) «On approval of the Rules for Procurement, Storage, Transportation and clinical use of donated blood and its components and on the invalidation of certain acts of the Government of the Russian Federation ». Moscow: RF Government; 2019

A prolongation of aPTT by 11% and PT by 8% was also observed, which was associated with a decrease in the activity of V and VIII factors [17, 18, 20].

The thromboelastography data for FFP and FLyP were found to be similar, which indicates the preservation of the hemostatic properties of the blood component after lyophilization [18]. FDP humidity did not exceed 2%. The lyophilizate is dissolved in 200 mL of water for injection in less than 6 min. The pH value of the rehydrated hemocomponent is alkaline-shifted equaling about 8. The shelf life is limited to 2 years at room temperature [4, 17, 18]. Regarding the stability of FLyP, this product was found to be most susceptible to changes in the activity of VIII and V factors, as well as the concentration of fibrinogen, at an elevated ambient temperature of 38–53 °C [14].

In the process of obtaining FDP LyoPlas N-w, a 21.6% decrease in the activity of VIII factor was observed (to a level of 0.79 ± 0.12 IU/mL). In comparison with the study results of French FDP, no changes in the factor V activity during lyophilization was noted, with the value of 1.07 ± 0.08 IU/mL being obtained. At the same time, a 25% decrease in the activity of the von Willebrand factor was shown, which was not evaluated in the FLyP study. The glycoprotein structure remained intact before and after lyophilization, which indicates the preservation of the function of the primary link of hemostasis. The remaining parameters varied in the range of 5.1–11.1% and corresponded to the physiological norm. A decrease in the factor VIII activity led to a prolongation of aPTT by 12.8%. Data on changes in PT was not provided. When LyoPlas N-w was rehydrated in 200 mL of water for injection, the dissolution time did not exceed 10 min [20, 47, 48]. The pH value of the rehydrated blood component was 7–7.2 [10]. After recovery, the product is recommended for use within 6 h. The shelf life of LyoPlas N-w is 15 months at a storage temperature 2–25 °C [5, 20, 48]. The results of a study of the safety limits of LyoPlas N-w under extreme conditions showed the stability of the blood component under a short-term temperature increase to 50 °C [48].

Highly limited information is available on the effect of lyophilization on the hemostatic properties of Bioplasma FDP. This product is known to have an efficiency profile similar to that of FFP. Bioplasma FDP is available in doses of 50 mL and 200 mL and is reconstituted with water for injection. The dissolution time does not exceed 10 min. The shelf life is 2 years at a temperature not exceeding 25 °C [6–8, 20].

The study of OctaplasLG Lyo showed a 30% decrease in the activity of factor VIII compared to FFP, as well as significantly lower protein S safety than for FLyP and LYOPLASN-w (a 34% decrease in activity). The remaining parameters of coagulation potential, including the activity of factor V and von Willebrand factor, varied within 7–19%. The most stable parameters were fibrinogen, factors X, XII, XIII, and protein C. The hemostasis system parameters were within the reference ranges established for blood plasma. No significant changes in PT and aPTT during lyophilization were recorded. The parameters of OctaplasLG Lyo thromboelastometry are comparable to the parameters of FFP. Other quality parameters met the requirements of the specification, including osmolarity — 333–350 mOsmol/kg; pH — 7.4–7.6; protein content — 55 mg/mL; humidity — no more than 1%; dissolution time — no more than 15 min. Since the production of OctaplasLG Lyo involves the introduction of excipients of citric and phosphoric acids, concentrations of citrate and phosphate ions of 20 mmol/L and 5.3 mmol/L, respectively, were additionally determined. These concentrations were higher than the similar values for FFP (16 mmol/L and 3.3 mmol/L, respectively). The identified deviations were recognized as acceptable, provided that the compliance of the quality and safety parameters with the established requirements was confirmed. The glycine content was determined at a level of 5 mg/mL. In general, the conclusion was made about the comparability of OctaplasLG Lyo and FFP quality profiles. The shelf life of the blood component is 2 years at room temperature storage [20, 24].

According to the results of quality control studies of pilot-scale FDP series developed in Belarus, compliance with the requirements of the internal specification was established. Coagulation potential was studied (II, V, VII, VIII, IX, X, XI, and XII factors, protein C, antithrombin III and α_2-antiplasmin, PT, aPTT). The activity of factor VIII was found to be 0.82 IU/mL, the other coagulation factors were 0.66–0.83 IU/mL, natural acticoagulants — 83–99%, and fibrinogen content –2.51 ± 0.25 g/L. Data on changes in the parameters during lyophilization are not provided [21].

In an *in vitro* experiment, when adding FDP to the blood of patients with acquired coagulopathy, normalization of thromboelastometry parameters was shown. This indicated the potential clinical effectiveness of the blood component [22]. The conducted assessment of the FDP physicochemical properties found its humidity ranging 0.58 ± 0.3%, osmolarity — 284.1 ± 29.2 mOsmol/kg, and the total protein content — 53 ± 2 g/L. It was shown that the content of citrate ions, calcium, sodium, and potassium did not exceed the reference ranges [21]. According to the results of pyrogenicity and abnormal toxicity tests, FDP was recognized as safe [22].

When studying the FDP properties obtained without the addition of protective agents (Institute of Pharmacology and Toxicology of AMMS, China), a decrease in the activity of factor V by 19.3%, factor VIII by 21.4%, and von Willebrand factor by 26.5% was noted; despite this, the values of the parameters corresponded to the physiological norm. The activity of factors II, VII, IX, X, XI, XII, plasminogen, antithrombin III, α_2-antiplasmin, protein C, and protein S decreased by no more than 5% during lyophilization [25]. To increase the coagulation potential, mannitol was introduced into the plasma at a

concentration of 25 g/L and the pH of the solvent (water) was adjusted to 7.3–7.4 with a phosphate-buffer saline (First Medical Center of PLA General Hospital, China). This made it possible to increase the safety of factors V and VIII by 12% and 18%, respectively, and to achieve their activity in FDP of more than 0.8 IU/mL. The residual moisture content of the dry blood component did not exceed 2%, and the recovery time with water for injection was 13 min [26].

Studies of a new generation FDP product developed by Teleflex Inc. (USA) using polymer containers with a membrane demonstrated a slight decrease in fibrinogen content within 7%, factor V activity within 15%, factors VIII and von Willebrand within 10%, as well as protein C and protein S within 9% and 7%, respectively. The decrease in the activity of other blood coagulation factors did not exceed 16%. Prolongation of PT to 12.9 s (by 7%) was noted. All parameters of the coagulation potential of FDP were in the range of reference values. The revealed differences did not exceed the threshold of bioequivalence of FDP with FFP — 20%. The experimental samples were characterized by humidity of the order of 1%, protein content of at least 50 g/L, osmolarity of 298.1 ± 7.2 mOsmol/kg, pH of 6.9 ± 0.2, and recovery time with water for injection of 1 min. According to the results of stability assessment, it is recommended to store FDP for no more than 3 years at a temperature 2–8 °C and for several months at room temperature [20, 30].

In the process of obtaining a similar product manufactured in the USA and Canada using the Terumo BCT Biotechnologies technology, factor VIII turned out to be the most susceptible to inactivation with its activity during lyophilization decreasing by 12.8–14.8%. A decrease in the concentration of α_2-antiplasmin by 14.3% and protein S by 12.1% was observed. Changes in other coagulation parameters (fibrinogen, protein C) were absent or ranged 2.2–8.7%. The aPTT and PT levels increased by 4.9% (to 29.4 ± 2.5 s) and 4.1% (to 11.3 ± 0.7 s), respectively. In general, the changes in coagulation potential observed during lyophilization did not exceed 20%; therefore, the FDP hemostatic properties were considered comparable to those of FFP. In addition, no deterioration in the parameters of thromboelastometry was established when comparing FDP with native plasma. The quality parameters of the dry blood component were within the normal range: humidity — less than 2%, total protein — more than 50 g/L. The dissolution time ranges within 5 min in water for injection. The values of osmolarity of 280.8 ± 12.8 mOsmol/kg and pH of 7.8 ± 0.1 were measured for the rehydrated blood component. Based on the results of the stability analysis, a shelf life of 2 years at room temperature was determined [20, 31–33].

In the Russian Federation, the following requirements for FDP safety parameters are set: humidity — less than 2%, total protein — more than 50 g/L, factor VIII activity — at least 0.5 IU/mL, sterility. The shelf life is 5 years at a temperature 2–20 °C[5]. The FDP produced using the Lyokon lyophilization technology exhibits the total protein content of 61.9 ± 3.6 g/L, the factor VIII activity of 0.56 ± 0.03 IU/mL, the fibrinogen concentration of 2.5 ± 0.2 g/L, aPTT of 79 ± 3 s, and PT of 23 ± 1 s. When comparing the plasma coagulation profile before and after lyophilization, a significant inactivation of factor VIII by 50% and prolongation of aPTT by 2.3 times were determined. Practically no changes in PT were observed [49]. When FDP was dissolved in 250 mL of 0.9% saline solution, moderate hyperosmolarity of the blood component at a level of 640 ± 22 mOsmol/L was noted [50]. A stability study after 3 months of storage in a hot climate with an increase in ambient temperature to 40 °C showed inactivation of factor VIII to 0.01 IU/mL and a significant decrease in fibrinogen content. During the same period at room temperature 20–25 °C, the activity of factor VIII fell below normal (0.46 ± 0.02 IU/mL). When stored in a refrigerator at 5 °C, it was at the lower limit of the regulated range and amounted to 0.49 ± 0.03 IU/mL [51]. Long-term stability tests are currently underway [50].

The presented results of studying the properties of FDP indicate the relevance of developing approaches to improving its coagulation potential. To increase the stability of FDP, it is possible to introduce lyoprotectors, such as glutamine, glycine, sucrose, trehalose, sorbitol, mannitol, or pH regulators [40, 52, 53]. To compensate for the pH value, it is possible to add bicarbonate buffer solution, citric or phosphoric acids to the native plasma, or saturate the dry blood component with purified CO_2 after the completion of the drying process [20, 24]. The possibility of using HEPES medium (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid), which is a high-capacity zwitterionic organic buffer at neutral pH values (pH = 7.55), was demonstrated [54]. In the presence of HEPES, the activity of factor VIII in FDP was found to increase by 12–18% compared to that in FDP obtained without the addition of stabilizers [12, 40]. Correction of the hydrogen index can also be carried out by restoring FDP with water for injection, acidified to a pH value of 1.5 ascorbic acid or citric acid [5, 7, 20]. Some authors recommend avoiding the use of glucose and other reducing sugars as lyoprotectors, which may interact with free amino acid residues during lyophilization, affecting protein properties [52]. It should be noted that when introducing excipients into the plasma, their harmlessness must be carefully proven. In the Russian Federation, it is currently possible to use only solutions and media approved in transfusion practice[6].

[5] Enactment of the Government of the Russian Federation No. 797 (dated 22.06.2019) «On approval of the Rules for Procurement, Storage, Transportation and clinical use of donated blood and its components and on the invalidation of certain acts of the Government of the Russian Federation ». Moscow: RF Government; 2019.

[6] ibid.

Use of freeze-dried plasma in extreme conditions

FDP is used in the provision of medical care in many countries. The FDP effectiveness for early transfusion therapy has been repeatedly confirmed in practice.

FLyP was used to provide transfusion assistance in military operations in the Sahel region of Central Africa, Djibouti, Afghanistan, and Iraq. Its clinical effectiveness has been studied in patients in intensive care units in Afghanistan. This product is approved in France for civilian use in extreme conditions [4–8, 17]. The United States also used FLyP in special military operations in Afghanistan and Iraq; since July 2018, its emergency use has been allowed [6, 10].

LyoPlas N-w has been used in medical institutions in Germany, by helicopter ambulance crews in the UK, Sweden, Norway, Finland, and Australia and, since 2012, by foot patrols in the UK. The safety and effectiveness of its use at the prehospital stage in the treatment of traumatized children has been proven [4–8]. Since 2013, the Israel Defense Forces has approved the use of FDP LyoPlas Nw at the pre-hospital stage. Currently, Israeli air and ground ambulances are equipped with LyoPlas Nw [5, 8, 48]. Military specialists in extreme medicine (physicians and paramedics) have two sets of AB(IV) FDP in their tactical vests [8].

Since 1996, Bioplasma FDP has been used in South Africa along with joint ventures to provide transfusion care to patients with blood loss resulting from trauma or postpartum bleeding [6–8].

CONCLUSION

The most promising areas of FDP production include quarantined or pathogen-reduced plasma, or a single-donor pooled blood component.

Plasma quarantine makes it possible to protect the patient from transmission of hemotransmissive infections. However, this is a rather lengthy process that takes at least 120 days. Pathogen reduction makes it possible to increase the infectious safety of FDP products and shorten the duration of their production for clinical use; at the same time, it can negatively affect their hemostatic potential. From the point of view of infection safety, it is preferable to use a single-donor product. At the same time, pooling makes it possible to standardize the blood component.

In extreme situations, the use of group AB(IV) FDP is of particular relevance due to the absence of the need to select a donor–recipient pair. This increases the efficiency of early transfusion therapy, which plays a key role in providing emergency medical care outside of hospital settings.

The currently known commercial FDP products are available in glass vials. The advantages of using FDP in polymer containers for providing transfusion care at the prehospital stage are obvious. Developments in this area are actively underway in the USA, Canada, and the Russian Federation. The use of membrane technology allows for a full cycle of blood component production in a single closed system while maintaining sterility.

The mass production of new-generation medicines in durable compact polymer containers is one of the ways to ensure uninterrupted transfusion care at the pre-hospital stage. This may increase the survival rate of the wounded and those with acute blood loss as a result of severe injuries in emergency situations. Therefore, creation of domestic technologies and consumable systems for plasma lyophilization, as well as development of approaches to improve the FDP effectiveness, are highly important tasks for Russian transfusiology.

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FUNCTIONING PRINCIPLES OF THE NETWORK OF BIOLOGICAL RISK MONITORING OF THE FEDERAL MEDICAL AND BIOLOGICAL AGENCY OF RUSSIA

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Introduction. The activity aimed at biological risk monitoring (BRM) ensures timely response against emerging biological threats in order to prevent their negative impact on human health. The improvement and further development of the existing network of BRM of the Federal Medical and Biological Agency (FMBA) of Russia requires understanding of its functioning principles.
Objective. Substantiation of the functioning principles of the BRM network in the entitled territories and organizations of the FMBA.
Materials and methods. The study was conducted using the automated information system of the FMBA Center for BRM, which aggregates BRM data from the territories and organizations serviced by FMBA. The methods of systems analysis, reverse engineering, classical logic, analysis, synthesis, comparison, generalization, categorization, and classification were used.
Results and discussion. A comprehensive study of the operating BRM network of FMBA was conducted. Its aims, objectives, functions, characteristics, and activities were examined. Using the method of reverse engineering, 19 key principles of the BRM network were substantiated. These principles were classified based on stratification of classes according to the types of activities that ensure the BRM network functioning as a complex organizational system. As a result, the principles were distinguished into informational and technological, organizational and managerial, and scientific and practical classes.
Conclusions. The functioning principles of the MBR network in the territories and organizations serviced by the FMBA were identified, formulated, substantiated, and classified. These include the use of the systems approach, the principle of continuous monitoring and reporting, the principle of comprehensive information and analytical support, etc. The results obtained can be used as the basis for decision making when optimizing the technology of BRM monitoring by FMBA.

Keywords: biological safety; biological risk monitoring; network functioning principles; biological risk monitoring network; systems approach

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ПРИНЦИПЫ ФУНКЦИОНИРОВАНИЯ СЕТИ МОНИТОРИНГА БИОЛОГИЧЕСКИХ РИСКОВ НА ТЕРРИТОРИЯХ И В ОРГАНИЗАЦИЯХ, ОБСЛУЖИВАЕМЫХ ФМБА РОССИИ

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Введение. Мониторинг биологических рисков (МБР) обеспечивает своевременное реагирование на возникающие биологические угрозы и предотвращение их негативного воздействия на здоровье человека. Для совершенствования и развития сети МБР ФМБА России необходимо знание и понимание принципов его функционирования.
Цель. Научное обоснование принципов функционирования сети МБР на территориях и в организациях, обслуживаемых ФМБА России.
Материалы и методы. Информационной платформой для исследования послужила автоматизированная информационная система Федерального информационно-аналитического центра мониторинга биологических рисков ФМБА России (ФИАЦ ММБР ФМБА России), агрегирующая данные МБР на территориях и в организациях, обслуживаемых ФМБА России. Исследование построено на применении научных методов системного анализа, обратного инжиниринга, классической логики, анализа, синтеза, сравнения, обобщения, категоризации и классификации.
Результаты и их обсуждение. Проведено всестороннее исследование действующей сети МБР ФМБА России. Рассмотрены и детально проанализированы цель, основные задачи, функции и направления деятельности созданной сети МБР, ее свойства, характеристики и особенности. На основании применения метода обратного инжиниринга обоснованы 19 ключевых принципов работы сети МБР. Разработана и представлена классификация перечисленных принципов, основанием для которой послужил признак разделения на классы по видам деятельности, обеспечивающей функционирование сети МБР как сложной организационной системы. В результате выделено 3 класса принципов: информационно-технологической, организационно-управленческой и научно-практической направленности.
Заключение. По результатам проведенного исследования были определены, сформулированы, обоснованы и классифицированы принципы функционирования сети МБР на территориях и в организациях, обслуживаемых ФМБА России. Среди них: системный подход, принцип непрерывности мониторинга и представления его результатов, принцип комплексности информационно-аналитического обеспечения и некоторые другие. Научное обоснование ключевых принципов, базирующееся на результатах исследования

процессов функционирования сети мониторинга, будет способствовать выработке предложений по оптимизации и совершенствованию технологии мониторинга биологических рисков ФМБА России.

Ключевые слова: биологическая безопасность; мониторинг биологических рисков; принципы функционирования сети; сеть мониторинга биологических рисков; системный подход

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INTRODUCTION

At present, the issues of biological safety are acquiring a greater importance and relevance. According to the experts of the Federal Medical and Biological Agency (FMBA) of Russia [1], the objective need to create an effective biosafety system is determined by the growing biological threats posed by various types of infections caused by pathogenic and opportunistic microorganisms (e.g., *Flavivirus*, SARS-CoV-2, MERS-CoV, and Ebola viruses), as well as ESKAPE pathogens (a group of multi-drug resistant bacteria, mostly responsible for nosocomial infections — *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.*). The antimicrobial resistance of these microorganisms has increased dramatically, having reached pandemic proportions [2, 3].

FMBA is responsible for the health and sanitary-epidemiological well-being of employees in more than 730 organizations with highly hazardous working conditions, including radiation, chemical, and biological risks. The FMBA provides support to more than 3.3 million people residing and working in 20 closed administrative-territorial entities, 39 satellite cities, and science cities in 59 Russian regions and on the territory of the Baikonur complex. FMBA is responsible for ensuring the biological safety and security of the personnel in the government-approved list of territories and organizations (referred to as entitled territories and organizations).

Activities in the field of biological risk monitoring (BRM) are aimed at timely response to emerging biological threats in order to prevent their negative impact on human health, including the following:

- identification, analysis, prediction, evaluation, and biological risk ranking based on unified criteria approved by the Russian government;
- accumulation of data and its implementation to assess the effectiveness of measures aimed at ensuring biological safety;
- development of measures to prevent and reduce biological risks, improve the protection of the population and the environment from the effects of dangerous biological factors, and mitigate biological threats.

In order to address these problems, the FMBA of Russia has established a BRM network, which has been operating since January 2022 in the entitled territories and organizations.

The growing amount of biological threats and the associated health risks determine the importance of analyzing the processes and functioning principles of the FMBA BRM network with the purpose of its optimization and further development based on the evidence-based approach. There is a lack of research studies that would substantiate the functioning principles of BRM technologies in general and the BRM network operated in the entitled territories and organizations of FMBA. These circumstances determine the relevance and novelty of this study.

In this article, we set out to develop a scientific basis for the functioning principles of the network of biological risk monitoring in the entitled territories and organizations of the FMBA of Russia.

MATERIALS AND METHODS

The study was based on Russian and foreign publications in peer-reviewed scientific journals presented in electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English languages (Web of Science, Scopus, PubMed, Google Scholar, Cochrane Library).

The FMBA database “Regulatory Legal Acts of Radiation, Chemical and Biological Monitoring” and the regulatory legal documents of the BRM network database (FMBA) were used to analyze the content of legal documents. The automated information system of the FMBA Center for BRM, which aggregates data on biological risks monitoring in the entitled territories and organizations of FMBA, served as the information platform for the study.

The study was conducted using the methods of systems analysis and reverse engineering [4], as well as general scientific methods, including classical logic, analysis, synthesis, comparison, generalization, categorization, and classification.

RESULTS AND DISCUSSION

The processes and operations of the FMBA BRM network were studied based on the data provided by the FMBA Information Analysis Center. The aims, objectives, functions, and activities of the established MBR network, as well as its properties, characteristics, and features, were thoroughly examined. In total, 19 key principles of the BRM network were identified, formulated, and justified. The FMBA BRM network is a complex organizational system, which functions based on operating principles.

Principle of the network architecture of biological risk monitoring

The organizational architecture of biological risk monitoring in the entitled territories and organizations of FMBA is a geographically and functionally distributed network of governmental institutions participating in the BRM network, consisting of subordinate medical organizations (177); microbiological research organizations (56); blood supply service institutions (17); territorial authorities (63), and other institutions of the FMBA of Russia (5) (Fig. 1).

As of today, the BRM network comprises 318 FMBA organizations, including six reference centers based at leading research and medical FMBA organizations, two of which have a federal status. Since January 1, 2022, the FMBA has established the Biological Risk Monitoring Information Analysis Center, which serves as the coordinating authority (coordinator) of the BRM network.

The FMBA BRM Information Analysis Center solves the following tasks:

- activity coordination of the BRM network within the established FMBA scope;
- collection and processing of information about biological threats in the entitled territories and organizations, as part of: medical activity; federal state epidemiological surveillance (supervision); state control (oversight) over the safety of donor blood and its components;
- continuous monitoring of biological risks using the information resources of the FMBA BRM Information Analysis Center, including identification, analysis, forecasting, evaluation, and biological risk ranking in accordance with unified criteria;
- prompt reporting to the FMBA management on the identified biological threats in the entitled territories and organizations;
- resource support monitoring of the FMBA medical organizations for carrying out diagnostic, preventive, medical, and rehabilitation measures in the event of biological threats in the entitled territories and organizations.

The structure of the Center includes two administrations: data collection and analysis, forecasting and assessment of biological risks. Taking into account the specifics of their activity, the following structural units have been formed (Fig. 2). Thus, the principle of BRM network architecture is implemented.

Principle of prioritization of compliance with the intended purpose

This principle is determined by the FMBA priority of protecting human life and health. The main purpose of establishing the network of biological risk monitoring was to create a reliable mechanism for monitoring biological risks in the entitled territories and organizations of FMBA, in accordance with Federal Law No. 492-FZ[1] and the relevant Decree of the Russian government.

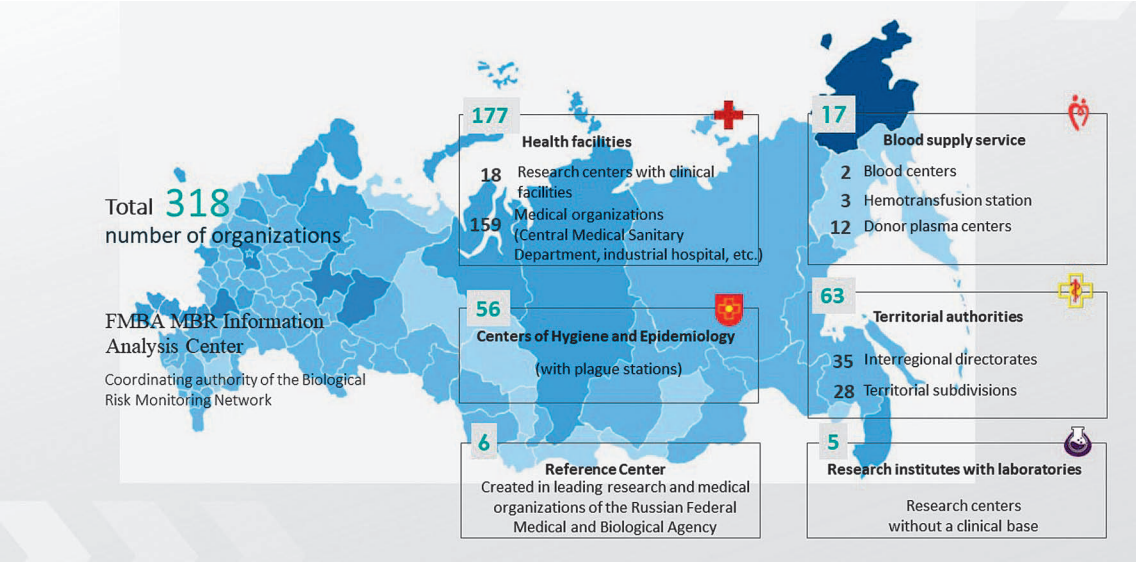

Figure prepared by the authors

Fig. 1. FMBA Biological Risk Monitoring network

[1] Federal Law No. 492-FZ dated 30.12.2020, "On Biological Safety in the Russian Federation".

Therefore, the main purpose underlying the organization of the BRM network was to ensure the biological safety of the FMBA personnel. In this regard, the principle of compliance with the intended purpose, or the target purpose principle, has been identified as one of the main functioning principles of the BRM network. All network activities are subordinated to this main purpose, which is given absolute priority: ensuring the biological safety of the FMBA work force, as well as protecting the environment from the effects of hazardous biological factors.

Principle of relying on a regulatory framework

Currently, the FMBA have developed regulatory legal acts that govern the process of biological risk monitoring in its entitled territories and organizations. The legal basis for the creation and operation of the BRM network is the Federal Law[2], Russian Federation Presidential Decree[3], Decrees of the Russian Government, departmental regulatory legal acts.

At present, the database of regulatory legal documents, created in the FMBA BRM Information Analysis Center, comprises 69 documents regulating the BRM network activities. These include Federal laws of the Russian Federation, Russian Federation Presidential decrees, resolutions and executive orders of the Government of the Russian Federation, departmental orders and instructions of the FMBA, departmental orders of the Ministry of Health of Russia, Sanitary Rules and Regulations, National State Standards, orders and instructions of the Centre for Strategic Planning of the FMBA, recommendations, instructions and methods of the FMBA MBR Information Analysis Center.

The current regulatory legal framework is the legal foundation for implementing state regulation measures in the field of ensuring biological safety and countering biological threats. This foundation determines the legal regulation of relations in the field of establishing, applying, and enforcing mandatory requirements for biological safety, including BRM, in the prevention and occurrence of natural and man-made dangerous biological situations of internal and external (cross-border) origin caused by natural and anthropogenic factors, as well as bioterrorist acts [5]. Thus, another essential principle of the MBR network is that of relying on a regulatory framework, or the regulatory regulation principle.

Principle of systems approach

This principle implies considering the BRM network as a system that combines a holistic set of interconnected elements [6]. The BRM network has the characteristics of a complex specialized organizational system, including the following:

- holism (the dependence of each element on its place within the overall network “organism”);
- primacy of the whole (subordination of the purposes of local elements to the main purpose of the entire system);
- connectivity (the presence of intra-system relationships between elements, including horizontal and vertical connections);
- structural properties (the ability to represent a system through the structure of connections and relations between elements);

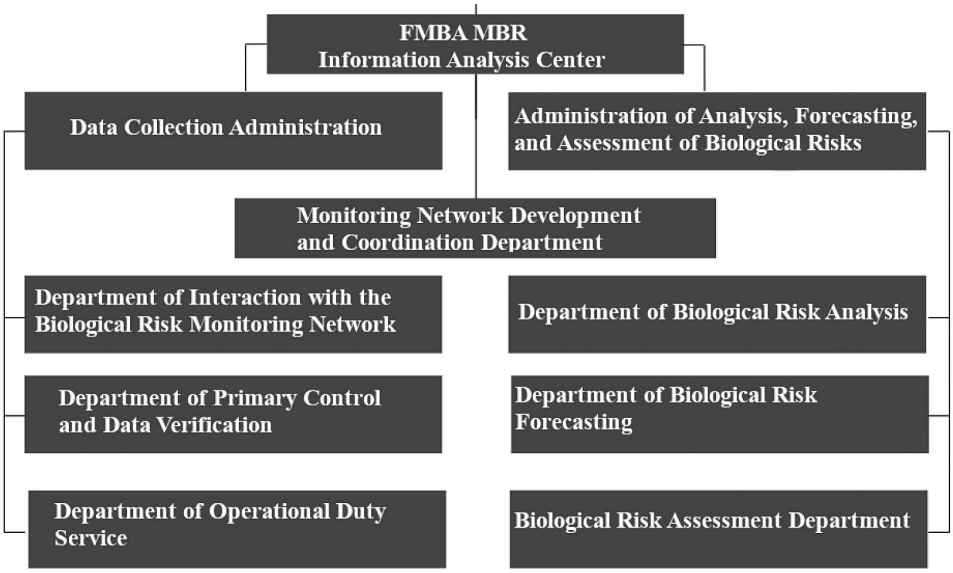

Figure prepared by the authors

Fig. 2. Structure of the FMBA Biological Risk Monitoring Information Analysis Center

[2] Federal Law No. 492-FZ dated 30.12.2020, “On Biological Safety in the Russian Federation”.
[3] Russian Federation Presidential Decree No. 97 dated 11.03.2019, “On the Fundamentals of the Russian Federation State Policy in the Field of Chemical and Biological Security for the Period up to 2025 and Beyond”.

- hierarchy/mono-centricity (subordination of the structural elements to a coordinator — FMBA BRM Information Analysis Center);
- synergy (functioning of interconnected elements such that generating qualitatively new properties of the system, which are not a mere sum of the properties of its elements) and some others.

The systems principle allows not only assessment of the integrity of the study object and the connections between its elements, but also determination of the sources and vectors of its development [7].

The formation of the BRM network as a system that functions both in planned and emergency modes required the determination of the object of this system's activities and the methodological approaches necessary for developing its multi-level, hierarchically subordinate structural and functional organization of elements covering the relevant functional spectrum of FMBA in the field of biological safety. A detailed analysis of the BRM network activity as a system is the subject of a separate study, which should include, among other things, the study of its properties, characteristics, and features, as well as the system's connections with the external environment.

Principle of continuous monitoring and its results reporting

The purpose-oriented activity of the BRM network, regulated by relevant legal documents, determines its continuous operation to implement its main tasks and ensure the biological safety of FMBA personnel. The geographical distribution of the entitled organizations and territories of the FMBA of Russia, which covers many time zones, necessitates the provision of round-the-clock data collection and processing, including continuous monitoring, screening, indication, identification, data verification, and etiological diagnostics of the quantitative and qualitative state of dangerous biological factors and risks to human health, including taking into account long-term negative consequences for the present and future generations.

An important component is the provision of timely information, presentation to consumers of the results of analysis and forecasting of biological threats and risks, operational analytical and forecast information for the organization of work to prevent, neutralize, or eliminate threats and risks to health [8]. Information users are the departments and management staff of the Centre for Strategic Planning of the FMBA of Russia, the interested agencies, the state information system in the field of ensuring biological safety (GIS BB). The main principle in this area of work is the principle of continuous monitoring and reporting of results.

Principle of interconnectedness of information flow management and data aggregation

Data aggregation is the process of combining and summarizing data from disparate sources into a single data set. The aggregation process prepares data for analysis, making it easier to understand the patterns of information flow [9].

The main tool of the FMBA BRM Information Analysis Center is its information system (IS), which is used for aggregation — collection and processing of data on biological threats and risks and formation of a single set of data. Currently, more than 3500 users are connected to the BRM IS. To date, the FMBA BRM Information Analysis Center is equipped with a modern powerful computing complex, which includes server equipment, memory blocks for data storage, high-speed communication channels. The existing computing complex allows achievement of the main tasks of BRM in the entitled territories and organizations of FMBA. It also allows for collecting and verifying any type of data.

Currently, the FMBA BRM Information Analysis Center receives data in four information streams. The first stream includes the submission of information by medical organizations based on the results of laboratory tests. The second stream includes data submitted by medical organizations based on the nosology of patients, including initial data. The third stream allows for the aggregation of data based on confirmed diagnoses through hygiene and epidemiology centers. The fourth stream includes information about identified outbreaks of infectious diseases and the respective measures taken.

The development of information technologies has led to a sharp increase in the speed and volume of data transmission. The structure of information exchange is changing significantly. The processes of data aggregation and information flow management require continuous technological monitoring, including their control and analysis, as well as the optimization of information streaming and aggregation.

Principle of complex automation of information processes and systems

Automation of information and technological processes and systems is the introduction and use of advanced information technologies and technical means that perform tasks and operations without direct human intervention [10].

The automation of the BRM network aims to solve the following tasks:

- reduction of monotonous routine work through the introduction of technologies for data replication, transmission, and interconnection, as well as the ability to automate and solve simple and complex tasks;

- expansion of the information and analytical capabilities of specialists, analysts, and managers through the creation of automated workplaces, the rapid and scientific processing of large and diverse data, including the use of artificial intelligence, neural networks, and hybrid methods;
- the ability to remotely obtain and share information, and to interconnect data from different sources;
- identification of the logic behind violations of sanitary rules and regulations.

Comprehensive automation includes the standardization and unification of equipment and software, the rational integration of information technologies into the existing workflow, ensuring the flexibility of the systems being created and the optimization of information processes, as well as the scalability and extensibility of the functionality of information systems and their resistance to failures.

The automated information system (AIS) of the FMBA BRM Information Analysis Center provides for the automatic input of data through the IP-interface, which ensures direct data collection from the information systems of medical and laboratory organizations participating in the BRM network. This approach ensures the prompt collection of up-to-date and reliable information.

For automatic operation of IS in identifying biological factors influencing changes in biological threats, the average annual morbidity rates (population in the entitled of FMBA) have been established as the threshold for informing. When the specified threshold is exceeded, measures are taken to analyze, predict, and assess biological risks. To ensure the effective operation of the automatic tracking system of biological threats (hazards), reference books of threshold values are systematically updated.

One of the main prospects for the automation development is integration with artificial intelligence and machine learning. To automate the monitoring of open sources of information about possible biological threats that can lead to an emergency, a subsystem based on AI technology has been developed and implemented at the FMBA BRM Information Analysis Center. This subsystem allows for rapid and high-quality content analysis of media news and social networks in order to identify information about a possible biological threat.

Principle of a friendly interface

The user interface is an important component of any automated, human-oriented system. A user-friendly (UF) interface refers to the intuitive means by which a user interacts with information systems, including data transmission systems. A UF interface should have a minimalist design and a high data loading speed.

In the information system of FMBA BRM Information Analysis Center, special electronic forms are used to interact with the BRM network participants. These forms are designed to meet the specific needs of medical organizations, hygiene and epidemiology centers, district medical centers, and territorial authorities. For specific events, data “showcases” and specialized user “windows” are developed and implemented for participants in planned training sessions.

Principle of import substitution for software products and hardware

An important technological feature of the present day is the need to switch to domestic information technologies and software and hardware tools in order to create a reliable domestic alternative to foreign analogues. Currently, the FMBA BRM Information Analysis Center carries out a transit to a domestic operating system that provides the required degree of information security and thereby reduces the vulnerability of software tools.

Principle of effective management of network activities

Various management levels of intra-network units and structures, as well as external stakeholders and/or parties in the monitoring process, are involved in informational, functional, and organizational cooperation within the BRM network [11]. These include:

1. Internal network elements: subordinate FMBA organizations — network participants and reference centers; structural and functional divisions of the FMBA BRM Information Analysis Center, including information and analytical units for data collection, processing, control, and verification; AIS information technology support units; network coordination and development units; operational duty service; contact center; the units for ensuring the activities of the FMBA BRM Information Analysis Center and the network development; training units, etc.
2. External information users, suppliers, and sources of information: administration of the Centre for Strategic Planning of the FMBA, the Center for Operational Management of the FMBA, FMBA Operations Control Centre, FMBA executive team, authorities (Emergency Control Ministry, Ministry of Health, Russian Federal State Agency for Health and Consumer Rights, Ministry of Defense, Ministry of Industry and Trade, etc.), institutions, information and scientific centers, the State Information System for Biological Security in the Russian Federation, Internet resources, etc.
3. FMBA work force, represented by the organizations served by FMBA, and the population residing in the territories served by FMBA.
4. External management structures: the Centre for Strategic Planning of the FMBA, the FMBA, the Government and the President of the Russian Federation.

A general structural diagram of the informational, functional, and organizational interaction of the BRM network is presented in Fig. 3.

As can be seen from Fig. 3, the informational, functional, and organizational interaction of the BRM network is a complex multidisciplinary process that requires effective management. This process management and its work coordination are entrusted to the FMBA BRM Information Analysis Center, which is the BRM network coordinator. Functioning of the BRM network is provided in two modes:

1) the mode of daily activities with the implementation of procedures for identifying, analyzing, forecasting, and evaluating biological factors;

2) emergency response to biological threats that could lead to an emergency situation.

In order for the BRM network management system to function successfully, it is necessary to continuously analyze changes in the external and internal environment and adapt actions to new conditions.

Subordination principle

The principle of subordination (co-ordination) implies management, within the framework of which the following processes are implemented: *vertical ordering*, i.e., vertical subordination of network elements, where one of the interacting elements acts as a leader, determining the activities of other participants in the relationship; *horizontal ordering* of business relations, where interaction between participants of the same level is established. Within the BRM network, the role of such a leading body belongs to the network coordinator — the FMBA BRM Information Analysis Center, which manages the activities of network participants. In turn, the FMBA BRM Information Analysis Center is part of the structure and subordinate to the Centre for Strategic Planning of FMBA. Medical support organizations are subordinate to the district FMBA centers. At the same time, all participants and the network coordinator, together with the Centre for Strategic Planning of FMBA, are subordinate to the FMBA and are obliged to comply with all directives, orders, and instructions of its structural divisions in accordance with those appointed by the head of the functional subordination Agency.

Since June 17, 2024, in accordance with the Decree of the President of the Russian Federation[4], the President of Russia is in charge of the FMBA activities.

Thus, the subordination vertical for the BRM network can be represented as follows: “President of Russia — FMBA — Center for Strategic Planning of FMBA — FMBA BRM Information Analysis Center (coordinator) — BRM network participants”.

Principle of permanence operational and duty support

The process of continuous monitoring of biological risks and the presentation of its results, together with the principle of effective management of biological risks, is implemented in the network, including through the activities of the operational duty service established at the FMBA BRM Information Analysis Center. The shifts of this structure operate around the clock and consist of operational duty officers and specialists who take over during daytime hours. Every day, the operational duty service provides the FMBA management with a report on the epidemiological situation in the entitled territories and organizations of FMBA. This is how the principle of permanent operational support is implemented in the BRM network.

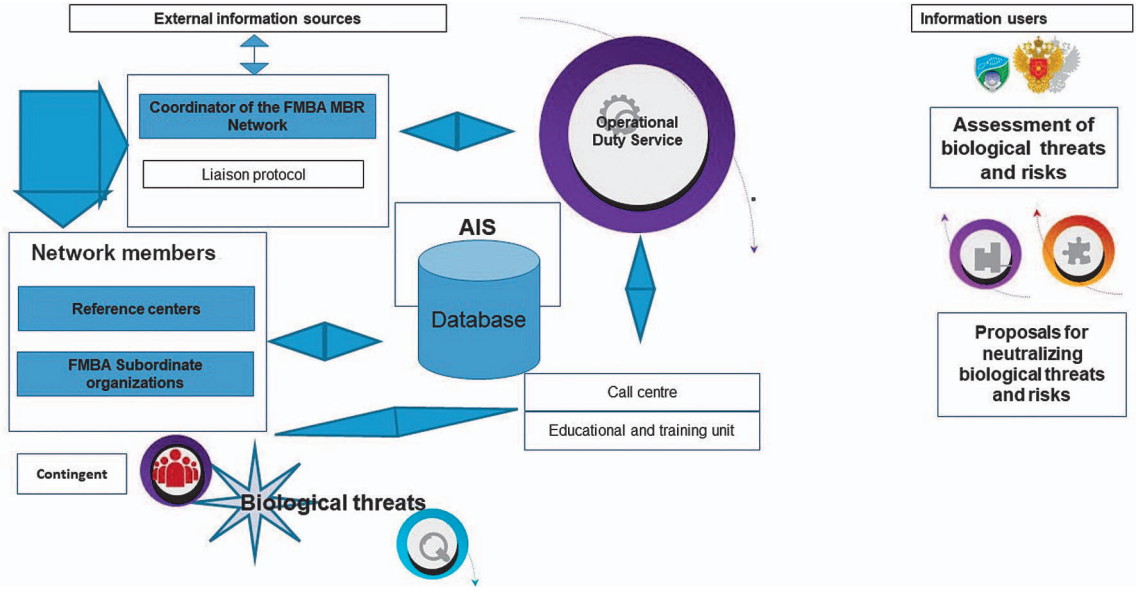

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Fig. 3. Structural scheme of informational, functional, and organizational interaction of the BRM network

[4] Decree of the President of the Russian Federation No. 522 dated 17.07.2024 “On the Federal Medical and Biological Agency”.

Principle of comprehensive information and analytical support

According to the Federal Law[5], biological risk monitoring includes the identification, analysis, forecasting, assessment, and ranking of biological risks based on unified criteria. This includes the information and analytical activities that are implemented in the FMBA BRM network.

Medical network organizations collect and perform initial analytical processing of medical and biological information, which is then forwarded to the FMBA BRM Information Analysis Center IS. The FMBA BRM Information Analysis Center conducts daily analysis of epidemic incidence, new, rare, recurrent, and spontaneous infections, changes in the properties and forms of pathogens, diseases associated with disruption of the normal human microbiota, infections associated with medical care (during accidents and terrorists attacks) at facilities where pathogens are used, the spread of drug resistance, and immunodeficiency conditions in humans.

The FMBA BRM Information Analysis Center conducts primary data verification and biological risk analysis, resulting in the receipt of structured information arrays on individual biological threats (hazards). It also determines methods for predicting biological risks. In accordance with the established criteria, the FMBA BRM Information Analysis Center submits information about identified biological threats (hazards) to the reference centers of the BRM network, which verify information about biological threats, including the analysis of biomaterial samples within 72 hours.

At the next stage, biological risks are predicted, i.e., the probability of dynamic changes in the biological threat indicators and their quantitative and qualitative characteristics is assessed. The outcome of biological risk prediction is the acquisition of data necessary for assessing the harm probability associated with the biological threat identified during the assessment of biological risks. If necessary, a report is prepared on the biological threat identification, which is submitted to the FMBA central office for subsequent transmission to the state information system for ensuring biological safety (SIS BS). In 2024, the FMBA Center for Strategic Planning created a database referred to as “Scientific Forecasting Methods”, which includes descriptions, characteristics, and features of approximately 100 scientific methods. The presented stages of the activity of BRM experts and their collaboration in collecting, analyzing, forecasting, and evaluating specialized data related to biological threats and risks demonstrate the comprehensive nature of information and analytical support in the MBR network [12].

Principle of ensuring the quality of information provided

The control mechanism of the BRM network ensures that specialized data on all cases of diseases registered in medical organizations is provided in a timely manner and that the data is transmitted to the FMBA BRM Information Analysis Center information system. At the same time, the quality of the information provided is monitored, including its completeness, relevance, and accuracy. In addition, the control mechanism includes automated analysis and notification of detected errors, as well as verification of operational and reporting data on key indicators.

The following types of activity are carried out daily in the FMBA BRM Information Analysis Center to ensure the information quality: checking the completeness and timeliness of filling out questionnaires by medical organizations of the BRM network in a single data collection format; deleting erroneous entries in the information resource (duplicates of infectious diseases cases and biological threat report files); detecting repeated filling of electronic forms.

The obtained quantitative indicators are verified on a weekly basis against statistical reports received from medical organizations, territorial authorities, hygiene and epidemiology centers. The reliability of incoming information is an important factor affecting the BRM network functioning quality. Reliable information refers to information (reports, data) from a reliable source about events, facts, phenomena, and processes that are authentic, truthful, and evidence-based, eliminating any doubt [13]. In this regard, particular attention is paid to the process of verifying information about biological threats, which is carried out by the reference centers of the FMBA BRM network. The assessment of biological risks is carried out based on unified criteria for the types of biological threats (hazards) to human health and the levels of biological risk.

Further forecasting, assessment, and ranking of biological risks are carried out by the Center’s analysts only for biological threats that have been verified by reference centers. To calculate the biological risk level, the severity of harm to human health caused by dangerous biological factors and the causing harm likelihood are used. The result of biological risk assessment is the determination of its level: acceptable, significant, or critical, which is reflected in the relevant information and analytical documents and reports.

Principle of professional competence development

One of the important areas in ensuring the BRM network quality is the appropriate professional training and retraining of senior officials and all employees of the BRM network organizations as a whole, as well as their

[5] Federal Law No. 492-FZ dated 30.12.2020, “On Biological Safety in the Russian Federation”.

coordinated interaction [14]. As part of specialized professional training, the network coordinator, the FMBA BRM Information Analysis Center, holds monthly seminars, training sessions, and classes with the participation of employees from medical organizations, territorial authorities, hygiene and epidemiology centers, blood centers, reference centers, district medical centers, and scientific institutions.

The coordinator organized the work of the FMBA BRM Information Analysis Center contact center for interaction with organizations participating in the BRM network. The contact center processes phone calls, messages received through the VKontakte widget, and e-mail requests, and conducts sessions with members of the BRM network in the order of sending and reviewing requests.

Principle of effective communication

The activities of the BRM network involve a wide range of specialists with different professional backgrounds and qualifications from various fields of scientific knowledge and practical industries, including medical professionals, biologists, hygienists, analysts, engineers, and programmers in the IT and telecommunications industries, dispatchers and operators of operational services, consultants, training instructors, methodologists, inspectors, etc. They must clearly and unambiguously understand each other, using the same terms and concepts, which ensures smooth functioning of the BRM network as a unified, well-coordinated, and effectively developing system.

In order to improve the quality of internal corporate communications, it was decided to develop the terminological apparatus of the FMBA BRM network and create a departmental thematic glossary of terms and definitions of the network. To that end, a special algorithm for creating a thesaurus-type glossary was developed, and the subject area and terminology of the BRM network were researched and categorized. The typology of the subject area is presented in Fig. 4.

Currently, work is underway to develop the terminology system of the BRM network [15], form dictionary definitions, and create a glossary project.

Principle of integrated scientific support

Scientific support includes a range of scientific, scientific-technical, scientific-methodological, and other activities aimed at obtaining and effectively implementing new knowledge, techniques, and technologies related to the BRM network functioning in the fields of medicine (including hygiene, sanitation, epidemiology, etc.), biology, management, analysis, and forecasting of biological threats and risks, information technologies, professional training, and social and biological emergencies.

Currently, as part of the scientific support for the BRM network activities, research is being carried out with the participation of specialists and researchers from the Sysin Research Institute of Human Ecology and Environmental Hygiene, the State Research Center — Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency, etc.

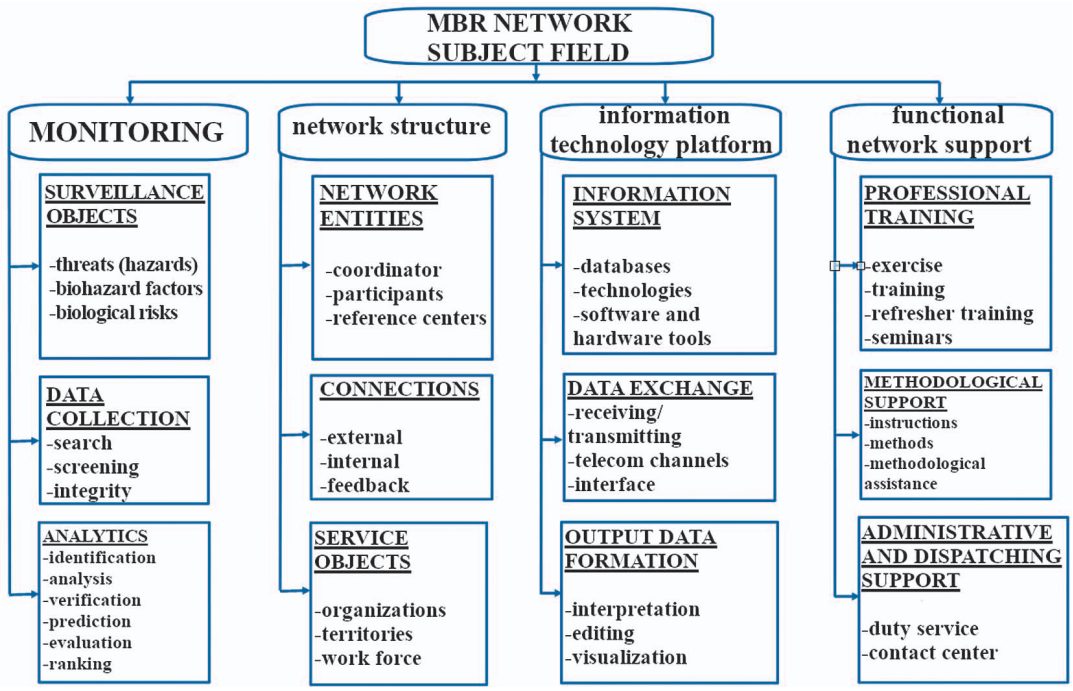

Figure prepared by the authors

Fig. 4. Typology of the subject area of the BRM network

Principle of targeted methodological support

Scientific support for biological risk monitoring includes providing targeted assistance to the BRM network participants in implementing functions that fall within their professional competencies and are related to the activity of the BRM network.

In order to improve cooperation and solve the problems of identifying and neutralizing biological threats and risks, the network coordinator develops methodological manuals, recommendations, instructions, booklets, and memos. Methodological assistance is provided to responsible specialists of the organizations participating in the BRM network on the issues of collecting and presenting information on biological threats (hazards). In accordance with the methodological recommendations, interdepartmental, regional, and facility-based exercises (trainings), classes, and gatherings are held, where qualified specialists from the FMBA BRM Information Analysis Center participate as instructors, methodologists, and intermediaries.

With the purpose of raising the professional competencies of the BRM network members working in the entitled territories and organizations of FMBA, the coordinator sends special teams (brigades) to provide targeted methodological assistance.

As part of the BRM network activity, a contact center has been established to provide daily advisory and methodological assistance to the network participants. To ensure its targeted nature, specific organizations have been assigned to each contact center employee. In 2024, the FMBA BRM Information Analysis Center contact center received 2549 requests from the FMBA organizations:1388 incoming calls, 1078 incoming emails, and 83 messages via the VKontakte widget; 34% of requests were completed with responses during the conversation, while the remaining 65% were completed within one working day.

Principle of sustainable development

Sustainable development is a controlled process aimed at developing society and nature, as well as production and other types of organizational systems. This also includes the network of biological risk monitoring, which, as noted above, refers to organizational systems. In a broad sense, sustainable development is aimed at preserving and ensuring favorable conditions for the life of current and future generations of people [16]. The concept of sustainable development is based on the following principles: ensuring an upward trend in development, ensuring a long-term character of development, and meeting the functional needs of the organizational system in both short- and long-term periods. These objectives are directly related to the network of biological risk monitoring.

When developing a concept for the sustainable development of the BRM network, it is advisable to implement standards and methodologies that can be used to monitor the functioning of the network itself and its subsystems (structural elements), as well as to qualitatively and quantitatively assess the target indicators [17].

Classification of the functioning principles of the BRM network

Following determination of the key functioning principles of the MBR network, they were subjected to classification. The classification feature was the type of activities that ensure functioning of the MBR network as a complex organizational system. Such a classification basis allows the overall set of fundamental principles established above to be divided into classes (groups) neither overlapping nor mutually excluding each other.

As a result, the following three classes of MBR network functioning principles were identified: (1) principles of information and technological orientation; (2) organizational and managerial principles; 3) principles of scientific and practical orientation. The distribution of principles by classes is presented in Fig. 5. When combined, the resulting classes form the initial set of principles.

CONCLUSION

As a result of the conducted research, the basic functioning principles of the MBR network in the entitled territories and organizations serviced of FMBA were identified, formulated, justified, and classified. The formed list of principles includes: a systems approach; the principle of compliance prioritization with the intended purpose; continuity of monitoring and presentation of its results; the principle of sustainable development; the complexity of information and analytical support, and some others. The reliance on these principles is one of the key factors that enable the biological risk monitoring network to function successfully, thus providing for the availability of the necessary resources and capabilities to identify and neutralize biological threats and to ensure biological safety.

The evidence-based substantiation of the key principles, based on the research of BRM network functioning, lays the foundation for the development of proposals for optimizing and improving the technology of biological risk monitoring by the FMBA of Russia, as well as for the development of a conceptual model for the functioning of the biological risk monitoring system.

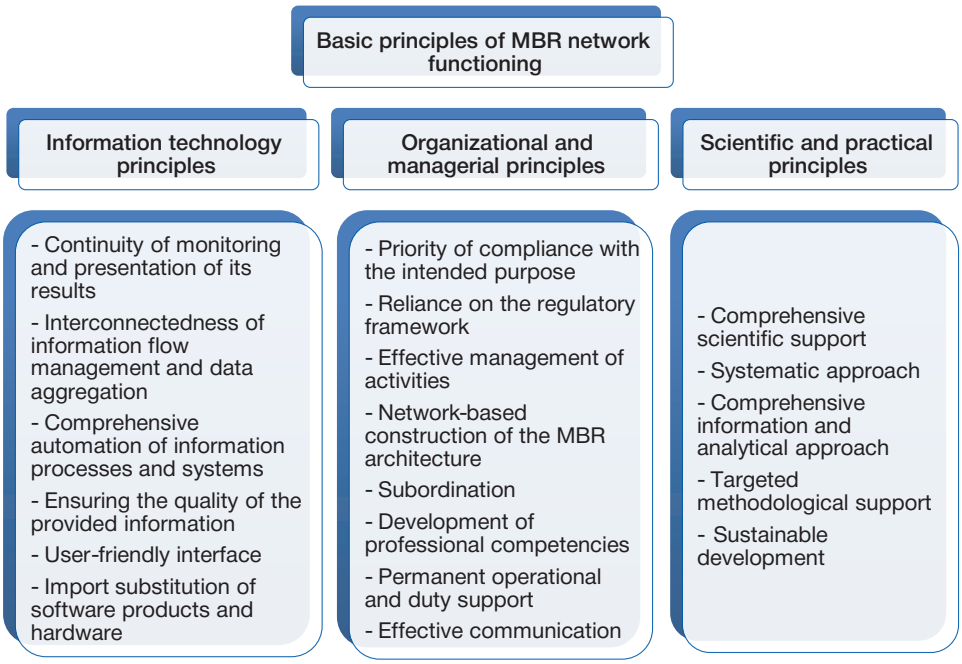

Figure prepared by the authors

Fig. 5. Classification of the basic functioning principles of the BRM network

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HEAVY METAL CONTENTS IN PLANTS GROWING IN THE RUSSIAN BALTIC COASTAL AREA

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Introduction. Prior to use in the production of food additives, ingredients, and biologically active substances, wild plants should be assessed in terms of heavy metal (HM) accumulation. This task is also relevant because wild plants can be consumed by the survived after accidents, disasters, or military operations at sea.
Objective. To assess the HM-related danger of coastal flora in the areas of potential landing of shipwrecked crews in the seas of the Russian Federation.
Materials and methods. The study objects were coastal algae and higher plants growing in the coastal area of the Gulf of Finland. Plant samples were collected in the Bolshoy Beryozovy Island, Hogland Island, and the Kurgalsky Peninsula. Prior to elemental analysis, the samples were dried at 80°C to a constant weight; their dry weight was estimated with an accuracy of 1 mg. The raw mass was estimated based on the dry weight data and the assumption that the water content in native tree leaves comprises 75%, in grass leaves — 85%, and in *F. vesiculosus* thalli — 70%. The dried material was mineralized by an MS-6 microwave sample preparation system (Volta, Russia). Elemental analysis was performed using an MGA-915M atomic absorption spectrometer. The measurement results were processed using the Statistica software.
Results. The Cu and Pb content in the studied plants was found to range within permissible limits. The permissible level of cadmium was exceeded by 2–4 times in *A. ptarmica*, *C. angustifolium*, and *U. dioica* on the Kurgalsky Peninsula, indicating the risk of food consumption. The minimum values of Mn content (less than 20 mg/kg of dry matter) were typical of two plant species (*L. japonicus* and *Salix* sp.) from the Bolshoy Beryozovy Island and *A. podagraria* from the Kurgalsky Peninsula. The toxic effects of Mn begin to appear when the daily intake exceeds 2 mg/day, while the maximum Mn content in the studied objects was 11.9 mg/kg. The high Zn content was typical of all plants on the Hogland Island, as well as *T. repens* and *A. podagraria* from the Kurgalsky Peninsula and *Salix* sp. and *L. japonicas* from the Bolshoy Beryozovy Island. The maximum amount of plant material that can be safely consumed was calculated to be approximately 0.17 kg/day of raw leaf mass.
Conclusions. The absence of daily intake limits for essential elements in regulatory documents makes it difficult to assess the severity of consequences of using plant raw materials for food and medicinal purposes and to apply a risk-based approach to assessing food safety. The high degree of danger associated with the use of plants from the Kurgalsky Peninsula (*A. ptarmica*, *C. angustifolium*, and *U. dioica*) is due to a significant excess of Cd limits. The Cu and Pb levels in all the studied plants was below the limits, indicating the absence of danger associated with these elements. The Zn content can be considered safe, since more than 1 kg of raw leaf mass must be consumed daily to meet the daily requirement, which is practically impossible in actual conditions.

Keywords: wild plants; wild plant-based food; micronutrients; heavy metals

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СОДЕРЖАНИЕ ТЯЖЕЛЫХ МЕТАЛЛОВ В РАСТЕНИЯХ ПОБЕРЕЖЬЯ БАЛТИЙСКОГО МОРЯ В РОССИЙСКОЙ ФЕДЕРАЦИИ

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Введение. Использование дикорастущих растений в производстве пищевых продуктов, добавок, ингредиентов и биологически активных веществ требует оценки сырья на содержание тяжелых металлов (ТМ). Это важно учесть и в связи с использованием дикоросов в пищу при выживании после аварий, катастроф или боевых действий на море.
Цель. Оценить потенциальную опасность употребления в пищу прибрежной флоры, способной аккумулировать ТМ, в местах возможной высадки экипажей судов, терпящих бедствие в акватории морей Российской Федерации.
Материалы и методы. Объектами исследования служили прибрежные водоросли и высшие растения, произрастающие на побережье Финского залива. Образцы растений собраны на участках побережий Финского залива: о-ва Большой Березовый, о-ва Гогланд, а также Кургальского п-ова. До проведения элементного анализа образцы всех растений досушивали при 80 °С до постоянного веса и оценивали их сухую массу с точностью до 1 мг. Оценку сырой массы осуществляли, опираясь на данные по сухой массе и условно принимая, что содержание воды в нативных листьях деревьев составляет 75%, в листьях трав — 85%, а в слоевищах *F. vesiculosus* — 70%. Минерализацию высушенного материала осуществляли в СВЧ-минерализаторе МС-6 («Вольта», Россия). Элементный анализ выполняли на атомно-абсорбционном спектрометре МГА-915М. Результаты измерений обрабатывали с помощью пакета прикладных программ Statistica for Windows 7.
Результаты. Содержание меди и свинца у изученных растений были в границах ПДУ. Допустимый уровень кадмия был превышен в 2–4 раза у *A. ptarmica, C. angustifolium* и *U. dioica* на п-ове Кургальский, что определяет риск использования их в пищу. Минимальные величины содержания марганца (менее 20 мг/кг сухой массы) характерны для двух видов растений (*L. japonicus* и *Salix sp.*)

с о-ва Березовый и для *A. podagraria* с п-ова Кургальский. Токсическое действие марганца (Mn) начинается при превышении нормы суточного потребления 2 мг/сут, в то время как максимальное содержание Mn у изученных объектов составляло 11,9 мг/кг. Высокое содержание Zn характерно для всех растений о-ва Гогланд, а также *T. repens* и *A. podagraria* с п-ова Кургальский и *Salix sp.* и *L. japonicas* с о-ва Большой Березовый. Было рассчитано предельное количество растительного материала, которое можно безопасно употребить в пищу; оно составило приблизительно 0,17 кг/сут сырой массы листьев.

Выводы. Отсутствие в нормативных документах ВДУ суточного потребления эссенциальных элементов затрудняет оценку тяжести последствий использования растительного сырья для пищевых и лекарственных целей и применение риск-ориентированного подхода в оценке безопасности питания. Высокая степень опасности использования в пищу растений п-ова Кургальский (*A. ptarmica, C. angustifolium и U. dioica*) обусловлена существенным превышением ПДУ по Cd. Содержание Cu, Pb во всех изученных растениях ниже ПДУ, т.е. опасность по этим элементам отсутствует. Содержание Zn является безопасным, поскольку для обеспечения суточной потребности в нем необходимо употреблять более 1 кг сырой массы листьев ежедневно, что в реальных условиях практически невозможно.

Ключевые слова: дикорастущие растения; пищевое применение дикоросов; микронутриенты; тяжелые металлы

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INTRODUCTION

The study of wild edible and medicinal plants combines two seemingly unrelated research directions: the search for safe food resources for human survival in nature and the development of innovative technologies for specialized and functional foods, food ingredients (food organic acids, enzymes, food and feed additives, biologically active substances, etc.). The use of wild plants is impossible without a related safety assessment of new types of plant-based food raw materials obtained from plants growing in areas that are not protected from pollutants produced by modern industries, energy facilities, and transportation vehicles.

Heavy metals (HM) are among the most dangerous agents that pollute the natural environment. It is customary to divide microelements into non-essential (not necessary for vital activity) and essential (microelements or indispensable nutritional factors). Excess amounts of even essential elements, such as zinc (Zn), copper (Cu), and manganese (Mn), have a toxic effect on living organisms, including humans [1].

The above problem necessitated the development of standards for physiological requirements for micronutrients, as well as the identification of maximum permissible levels (MPL) for their intake[1,2]. The methodological guidelines that establish the regulatory levels of micronutrients are revised on a constant basis; however, the daily requirements for Zn, Cu, and Mn remain unchanged at 12, 1 and 2 mg/day, respectively[3,4,5]. On the contrary, the MPL values for Zn and Mn were revised downward in 2004–2008 and, in the latest version of 2021[6], were excluded from the list of regulated parameters. This situation makes it difficult to assess the risks of using wild plants for food and medicinal purposes. However, paragraph 21 of the Food Security Doctrine of the Russian Federation calls for the continued harmonization of the characteristics and parameters of food quality and safety based on fundamental research in the fields of hygiene and nutrition science[7].

In the above connection, research efforts aimed at developing approaches to expert assessment of the risks of using wild plants for nutritional and (or) medicinal purposes due to their possible contamination with HM seem highly relevant. In the present study, we set out to assess the potential danger of consuming coastal flora in terms of HM accumulation, with a particular focus on the areas of potential landing of marine crews in distress in the seas belonging to the responsibility zone of the Russian Federation.

MATERIALS AND METHODS

Plant samples were collected along the coast of the Gulf of Finland, including the Bolshoy Beryozovy Island, located in the northern part of the Gulf, Hogland Island,

1 MR 2.3.1.2432-08 Standards of physiological requirements for energy and nutrients for various population groups in the Russian Federation. Moscow: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor; 2009.
2 MR 2.3.1.1915-04:2.3.1 Healthy eating. Recommended levels of food and biologically active substance consumption. Moscow: State Sanitary and Epidemiological Standards of the Russian Federation; 2004.
3 MR 2.3.1.2432-08 Standards of physiological requirements for energy and nutrients for various population groups in the Russian Federation. Moscow: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor; 2009.
4 MR 2.3.1.1915-04:2.3.1 Healthy eating. Recommended levels of food and biologically active substance consumption. Moscow: State Sanitary and Epidemiological Standards of the Russian Federation; 2004.
5 MR 2.3.1.0253-21 Standards of physiological requirements for energy and nutrients for various population groups in the Russian Federation. Moscow: Federal Service for Supervision of Consumer Rights Protection and Human Welfare; 2021.
6 Ibid.
7 Food Security Doctrine of the Russian Federation. Moscow: Rosinformagrotech, 2020.

located in the center of the water area, and the Kurgalsky Peninsula, which protrudes into the Gulf of Finland from the south and separates its Narva and Luga bays. The study objects were coastal algae and higher plants that grow in the coastal area of the Gulf of Finland. These plants have a long history of being consumed as food and are known for their medicinal properties:

Atriplex prostrata Boucher — hastate-leaved (spear-leaved) orache. It is widespread in the European part of the Russian Federation, in the Altai region, and in Eastern and Western Siberia. The leaves of this species contain vitamins A, E, P, PP, rutin, proteins, essential oil, fiber, and minerals. The leaves are a nutritious component of salads, hot and cold vegetable soups, side dishes, and omelets. The leaves are consumed before flowering. This species contains a set of substances with antioxidant and radioprotective properties [2].

Achillea ptarmica L. — pearl yarrow. In Russia, it is widespread in the European part, and, as an adventive plant, can be found in Western Siberia. The leaves have a tart, spicy taste with a slight bitterness, as well as a pleasant herbal aroma. The infusion reduces appetite and lowers blood glucose levels. Fresh herbs are added to ready-made dishes. The study of the phytochemistry and biological activity of substances extracted from different species of the *Achillea* genus has shown the prospects of their use in the food and pharmaceutical industries [3].

Aegopodium podagraria L. — bishop's goutweed. It is widespread in the European part of the Russian Federation, except for the Far North. Young light green leaves are edible. By autumn, the vitamin C concentration increases, sometimes up to 60–100 mg. It contains fiber, malic and citric acids, choline, beta-carotene, flavonoids, coumarins, mineral salts and essential oils, in noticeable quantities of iron, magnesium, potassium [4]. In terms of antioxidant activity, extracts of *Aegopodium podagraria* L. are superior to those of other studied species [5].

Chamaenerion angustifolium L. — narrow-leaved fireweed. It is widespread in the cold and temperate zones of Russia, including the Caucasus, Siberia, and the Far East, where it grows primarily on sandy and loamy soils. Fireweed tea is rich in iron, copper, potassium, and calcium. The above-ground mass contains 18.8% protein, 5.95% fat, 50.44% nitrogen-free extractive substances, 16.62% fiber, 8.14% ash, 0.75% calcium, and 0.43% phosphorus [6].

Fucus vesiculosus L. — bladderwrack. In the marine waters of the Russian Federation, it grows abundantly in the tidal zone of the White Sea, the southern part of the Barents Sea, and the western regions of the Baltic Sea, including the coast of the Hogland Island. It is used for making salads and as a fiber-rich additive to sea fish. Currently, it is considered a promising source of biologically active substances [7].

Lathyrus japonicus Wild — beach vetchling or sea pea. It is widespread in the northern territories of the Russian Federation and in areas with a temperate climate. It is a conditionally edible plant. It is not recommended for regular consumption due to the presence of oxalyldiaminopropionic acid (ODAP), which has neurotoxic properties. The aerial parts of the plant (stems, leaves, and seeds) are used for food purposes, and decoctions are used to treat cardiovascular diseases [8]. Beach vetchling is rich in vitamins (A, B_1, B_2, B_3, B_4, B_6, B_7, B_9, and C) and minerals (sulfur, chlorine, phosphorus, potassium, calcium, sodium, magnesium, titanium, nickel, cobalt, silicon, boron, molybdenum, selenium, manganese, copper, zinc, iodine, and iron).

Polygonum aviculare L. — dooryard knotweed. It is widespread in the Russian Federation, with the exception of the Arctic. Young leaves can be used in salads, soups, and stews. The extracts of this plant have been studied for their antioxidant, anti-inflammatory, antimicrobial, antitumor, and antidiabetic properties [9]. The infusion of this herb is used in traditional medicine as an anti-inflammatory agent due to its ability to remove kidney and bladder stones. It is also used as a hemostatic, hypotensive, diuretic, and astringent agent. It is included in herbal preparations used for chronic gastritis, stomach ulcers, bronchitis, kidney stones, uterine bleeding, cystitis, pulmonary tuberculosis, and other diseases [10]. Moreover, in comparison with other *Polygonum* species, knotweed extracts have the greatest potential in terms of their pharmaceutical effects on the kidneys and urinary tract [11].

Plantago media L. — hoary plantain. It is widespread in the European part of the Russian Federation and in various regions of Siberia. Young leaves are rich in fiber, flavonoids, polysaccharides, vitamins, and minerals, making them a recommended food for vegan and vegetarian diets as addition to cereals, sauces, smoothies, juices, and other beverages. Young leaves can be eaten raw (added to salads or cold soups), boiled, or canned [12].

Salix L. — willow. It is a plant genus that includes about 350 species. They are widespread in the Russian Federation from the subtropics to the Arctic and from the western borders to the east, including Kamchatka and Primorsky Krai. Willow leaves, which are a source of fiber, plant protein, organic acids, and vitamin C, can be boiled or consumed without heat treatment, but after being mashed and then fermented for 8–12 h [13].

Trifolium repens L. — white clover. It is ubiquitous in the Russian Federation. Fresh leaves of young plants are added to vegetable salads, soups, and stewed side dishes for meals of vegetables, meat, and seafood. In a dried and crushed form, fresh leaves are used in the manufacture of sauces, cheeses, and bakery flour. The results of research into the anticholinesterase and antiradical activities of *T. repens* extracts indicate their applicability in the treatment of neurodegenerative diseases [14]. It was found that clover flavonoids reduce intracranial and arterial pressure, relieve dizziness, improve hearing, and reduce tinnitus [15].

Urtica dioica L. — stinging nettle. In Russia, it grows in the European part and Western Siberia, and has been introduced to Eastern Siberia and the Far East. Young shoots and fresh leaves are used after boiling to make vitamin-rich green salads. The shoots and leaves are also used to make soups. Leaf puree is used to make omelets and casseroles. Stinging nettle leaves can be salted, pickled, or marinated. *U. dioica* is a medicinal plant that is widely used in various countries to treat hypertension [16]. It is capable of reducing glucose levels and regulating blood lipid levels, as well as exhibiting anti-inflammatory and antioxidant effects [17].

Sample plants were collected in an open sea area, simulating a survival situation on the coast, where help can only be expected from the sea. The species of terrestrial plants were identified in the areas where they were collected using the Plant Identification Guide for the Leningrad Oblast [18].

Young leaves from the apical part of the shoot were used for analysis. The collected leaves of higher plants were placed between sheets of ashless filter paper and dried in a plant press. Before placing the fragments of the brown alga *F. vesiculosus* in the plant press, moisture was removed from the fragments using ashless filter paper. Prior to elemental analysis, all plant samples were dried at 80°C to a constant weight; their dry weight was estimated with an accuracy of 1 mg. The raw mass estimation was carried out indirectly, based on the dry weight data and assuming that the water content in native tree leaves is 75%, in grass leaves — 85%, and in *F. vesiculosus* thalli — 70%. The dried material was mineralized by an MS-6 microwave sample preparation system (Volta, Russia) using the standard procedure [19]: in three stages, with a temperature and pressure increase from 120°C and 15 atm to 180°C and 25 atm. The total process time lasted 12 min.

Elemental analysis was performed using an MGA-915M atomic absorption spectrometer (Lumex, Russia) at a wavelength of the studied element spectral line, using certified reference materials (CRMs) for elemental analysis[8]. The content of all elements was identified by parallel measurements of the same mineralized samples.

The measurement results were processed using the Statistica softwire package. Sample collections were formed by combining plant samples based on their species and collection points. The sample size (4–6 specimens) was determined by the availability and material quantity at the point of debarkation. The distribution type of the sample collections was assessed using the Shapiro–Wilk test, which ultimately determined the use of parametric methods. The results include the mean values with confidence intervals for a significance level of $p = 0.05$. Based on the latter, the values obtained by direct measurements were rounded to three significant digits. The data on the element content in the raw mass, which is the result of converting the initial values on dry weight basis, contains the same number of digits after the decimal point as the initial values.

RESULTS AND DISCUSSION

The study of heavy metal accumulation, plant groups without significant differences between the members of relevant samples were determined. For example, a high content of manganese (more than 40 mg/kg of dry weight) was typical of three plants (*L. japonicus*, *A. prostratum*, *P. aviculare*) growing on the Hogland Island, and three (*P. minuta*, *A. ptarmica* and *U. dioica*) collected on the Kurgalsky Peninsula. Minimum values of manganese content (less than 20 mg/kg of dry weight) were typical of two plant species (*L. japonicus* and *Salix* sp.) from the Bolshoy Beryozovy Island and for *A. podagraria* from the Kurgalsky Peninsula. The mean manganese content (less than 40 mg/kg, but more than 20 mg/kg) was identified in the *F. vesiculosus* thallus and plants (*Salix* sp., *C. angustifolium*, *T. repens*) from the Kurgalsky Peninsula, as well as *C. angustifolium* from the Bolshoy Beryozovy Island (Table 1).

Plants were divided into two groups based on their zinc content. Its high content (more than 30 mg/kg of dry weight) was typical of all plants of the Hogland Island. This category also includes *T. repens* and *A. podagraria* from the Kurgalsky Peninsula, as well as *Salix* sp. and *L. japonica* from the Bolshoy Beryozovy Island. In the remaining plants, the Zn content was about 20 mg/kg of dry weight, showing no significant differences.

The highest lead content (over 0.4 mg/kg) was found in *L. japonicus* from the Hogland Island and in *P. aviculare* from the Bolshoy Beryozovy Island, as well as in *A. ptarmica* and *U. dioica* collected on the Kurgalsky Peninsula. In six plants, lead was either not detected (*A. prostratum*, *T. repens*, *A. podagraria*), or its content was estimated as low, no more than 0.1 mg/kg (*F. vesiculosus*, *P. minuta*, and *Salix* sp. from the Bolshoy Beryozovy Island). In the remaining three plants, the element level ranged within 0.2–0.4 mg/kg (*Salix sp*. from the Kurgalsky Peninsula and *C. angustifolium* from both habitats), i.e., they contained a mean concentration of the element.

It was difficult to compare plants in terms of their copper and cadmium contents due to the significant variation in the data in the studied sample collections. However, the mean values used to compose a descending order of element contents in plants (Table 2) showed that copper consistently ranks third, surpassed only by the lead content in *P. aviculare* and *L. japonicas* from the Bolshoy Beryozovy Island. The cadmium content was almost always lower than the lead content. The predominant sequences in the descending order of elements are as follows: $Mn \geq Zn > Cu > Pb \geq Cd$.

[8] M 04-64-2017. Food products and food raw materials. Feeds, compound feeds, and raw materials for their production. Method for measuring the mass fraction of cadmium, arsenic, tin, mercury, lead, and chromium using atomic absorption spectroscopy with an MGA-915, MGA-915M, MGA-915MD, or MGA-1000 atomic absorption spectrometer with electrothermal atomization. S. Petersburg; 2017.

Table 1. Elemental content in plants from different locations

Location	Plant	Elemental content, mg/kg of dry weight (upper values), mg/kg of wet weight (lower values)				
		Mn*	Zn*	Cu▪	Pb▪	Cd▪
Hogland Island	*A. prostratum*	46.7 ± 20.4	42.3 ± 9.3	0.327 ± 0.485	not detected	0.019 ± 0.013
		7.0 ± 3.1	6.3 ± 1.4	0.049±0.072	not detected	0.003 ± 0.002
	F. vesiculosus	37.3 ± 8.8	37.0 ± 11.2	1.66 ± 2.30	0.070 ± 0.046	0.306 ± 0.413
		11.2 ± 2.6	11.1 ± 3.4	0.49 ± 0.69	0.021 ± 0.013	0.091 ± 0.124
	L. japonicus	55.5 ± 9.4	69.3 ± 36.1	0.881 ± 0.931	0.506 ± 0.192	0.030 ± 0.025
		8.3 ± 1.5	10.4 ± 5.4	0.132 ± 0.139	0.076 ± 0.029	0.004 ± 0.004
	P. aviculare	79.2 ± 42.8	53.3 ± 30.4	0.073 ± 1.34	0.479 ± 0.158	0.007 ± 0.004
		11.9 ± 6.4	8.0 ± 4.6	0.011 ± 0.201	0.072 ± 0.024	0.001 ± 0.001
Bolshoy Beryozovy Island	*C. angustifolium*	30.6 ± 9.8	30.6 ± 9.8	1.99 ± 0.35	0.299 ± 0.042	0.106 ± 0.045
		4.6 ± 1.5	4.6 ± 0.3	0.30 ± 0.05	0.045 ± 0.006	0.016 ± 0.007
	L. japonicus	17.6 ± 3.5	47.2 ± 4.6	0.548 ± 0.025	0.585 ± 0.143	0.007 ± 0.002
		2.6 ± 0.5	7.1 ± 0.7	0.082 ± 0.003	0.088 ± 0.021	0.010 ± 0.000
	Salix sp.	17.2 ± 10.0	39.1 ± 7.1	0.544 ± 0.068	0.102 ± 0.021	0.025 ± 0.018
		4.3 ± 2.5	9.8 ± 1.8	0.136 ± 0.017	0.026 ± 0.005	0.006 ± 0.004
Kurgalsky Peninsula	*A. ptarmica*	57.4 ± 13.4	25.6 ± 2.6	2.26 ± 0.43	0.870 ± 0.324	0.870 ± 0.340
		8.6 ± 2.0	3.8 ± 0.4	0.339 ± 0.064	0.130 ± 0.049	0.130 ± 0.051
	A. podagraria	3.7 ± 3.5	34.1 ± 3.7	0.562 ± 0.410	not detected	0.010 ± 0.006
		0.6 ± 0.5	5.1 ± 0.6	0.084 ± 0.061	not detected	0.002 ± 0.001
	C. angustifolium	34.0 ± 4.6	21.1 ± 2.1	1.01 ± 0.43	0.213 ± 0.072	0.551 ± 0.231
		5.1 ± 0.7	3.2 ± 0.3	0.152 ± 0.064	0.032 ± 0.011	0.083 ± 0.035
	P. minuta	74.9 ± 12.5	21.2 ± 3.9	2.91 ± 2.06	0.040 ± 0.028	0.041 ± 0.029
		11.2 ± 1.9	3.2 ± 0.6	0.44 ± 0.31	0.006 ± 0.004	0.006 ± 0.004
	Salix sp.	31.2 ± 4.0	19.9 ± 7.1	2.09 ± 0.35	0.311 ± 0.049	0.054 ± 0.059
		7.8 ± 1.0	5.0 ± 1.8	0.52 ± 0.09	0.078 ± 0.012	0.013 ± 0.015
	T. repens	36.0 ± 7.8	42.7 ± 18.4	2.40 ± 1.78	not detected	0.007 ± 0.003
		5.4 ± 1.2	6.4 ± 2.8	0.36 ± 0.27	not detected	0.001 ± 0.000
	U. dioica	65.7 ± 28.2	22.9 ± 1.4	4.16 ± 0.82	0.440 ± 0.060	0.395 ± 0.107
		9.9 ± 4.2	3.4 ± 0.2	0.62 ± 0.12	0.066 ± 0.009	0.059 ± 0.016

Table prepared by the authors using their own data

Note: * — human daily element requirement[9] (mg): Mn = 2; Zn = 12; ▪ — element maximum permissible level[10] (mg/kg of wet weight): Cu = 5; Pb = 0,5; Cd = 0,03.

[9] MR 2.3.1.0253-21 Standards of physiological requirements for energy and nutrients for various population groups in the Russian Federation. Moscow: Federal Service for Supervision of Consumer Rights Protection and Human Welfare; 2021.
[10] Technical Regulations of the Customs Union. TP TC 021/2011 «About food safety» dated 9.12.2011. No. 880.

Table 2. Descending orders of elements in plants

Location	Plant	Descending orders of elements in plants
Hogland Island	*A. prostratum*	Mn ≥ Zn > Cu > Cd
	F. vesiculosus	Mn = Zn > Cu > Cd ≥ Pb
	L. japonicus	Zn ≥ Mn > Cu > Pb > Cd
	P. aviculare	Mn ≥ Zn > Pb > Cu > Cd
Bolshoy Beryozovy Island	*C. angustifolium*	Mn ≥ Zn > Cu > Pb > Cd
	L. japonicus	Zn > Mn > Pb ≥ Cu > Cd
	Salix sp.	Zn > Mn > Cu > Pb > Cd
Kurgalsky Peninsula	*A. ptarmica*	Mn > Zn > Cu > Pb = Cd
	A. podagraria	Zn > Mn > Cu > Cd
	C. angustifolium	Mn > Zn > Cu > Cd > Pb
	P. minuta	Mn > Zn > Cu > Pb = Cd
	Salix sp.	Mn > Zn > Cu > Pb > Cd
	T. repens	Zn ≥ Mn > Cu > Cd
	U. dioica	Mn > Zn > Cu > Pb ≥ Cd

Table prepared by the authors using their own data

Note: the decreasing orders of elements in plants are composed using the mean value and the standard error of mean ($M \pm m$).

In the group of plants from the Hogland Island, the closest Mn and Zn concentrations were observed, compared to other habitats. For example, two plants (*L. japonicus* and *Salix* sp.) from the Bolshoy Beryozovy Island and *A. podagraria* from the Kurgalsky Peninsula accumulated significantly more Zn than Mn.

The copper and lead content in all studied plants did not exceed the permissible limit[11]. The zinc and cadmium concentrations require our comment. Cadmium is highly toxic not only to animals and humans, but also to plants, including those used as food and pharmaceutical raw materials [20].

When the actual cadmium content was compared with the maximum permissible level (MPL), the cadmium level was 2–4 times higher in the plants of the Kurgalsky Peninsula (*A. ptarmica*, *C. angustifolium*, and *U. dioica*). In the leaves of the remaining 11 plants, the cadmium concentration was within the MPL, or the excess was not statistically significant. This also applies to the leaves of willow, the most well-known accumulator of cadmium [21, 22].

The zinc content in 1 kg of raw plant mass of all the studied plants was found to be below the daily requirement; thus, the dietary use of these plants is safe in terms of this element.

The regulatory documents of the Russian Federation do not regulate manganese concentration (MPL) in plants; therefore, the safety assessment of this element was carried out in accordance with the norms of physiological daily requirements[12], comprising 2 mg/day for manganese. Since the maximum content of manganese, noted in the studied plant objects, was 11.9 mg/kg, the reference to this value allowed us to determine the amount of plant material that can be consumed safely, namely up to 0.17 kg/day of raw leaf mass. However, the daily requirement for manganese does not coincide with the maximum permissible level, which requires knowledge of the excretion rate of this element from the body to assess its maximum safe daily intake. The MPL[13] value is 5 mg/day, which suggests that the safe consumption will be 0.42 kg/day. Unfortunately, the obtained value has to be recognized as unreliable, since it relies on data from a repealed regulation. In this regard, the issue of returning MPL to the number of regulated parameters becomes relevant.

[11] M 04-64-2017. Food products and food raw materials. Feeds, compound feeds, and raw materials for their production. Method for measuring the mass fraction of cadmium, arsenic, tin, mercury, lead, and chromium using atomic absorption spectroscopy with an MGA-915, MGA-915M, MGA-915MD, or MGA-1000 atomic absorption spectrometer with electrothermal atomization. S. Petersburg; 2017.

[12] MR 2.3.1.0253-21 Standards of physiological requirements for energy and nutrients for various population groups in the Russian Federation. Moscow: Federal Service for Supervision of Consumer Rights Protection and Human Welfare; 2021.

[13] MR 2.3.1.1915-04:2.3.1 Healthy eating. Recommended levels of food and biologically active substance consumption. Moscow: State Sanitary and Epidemiological Standards of the Russian Federation; 2004.

CONCLUSION

It can be stated that the absence of daily intake norms of essential elements in Russian regulatory documents makes it difficult to assess the consequences of wild plant consumption for food and medicinal purposes and to apply a risk-based approach in assessing food safety.

Manganese becomes a hazard factor at moderate daily intake doses of plant material provided that the level of daily requirement is exceeded.

A high-degree danger of consuming plants from the Kurgalsky Peninsula (*A. ptarmica*, *C. angustifolium*, and *U. dioica*) has been revealed due to a significant excess of the MPL for Cd. The Cu, Pb, and As content in all the studied plants is below the MPL, i.e., presenting no danger. The Zn content can be considered safe, since it is necessary to consume more than 1 kg of raw leaf mass daily to meet the daily requirement for this element, which is practically impossible in real survival conditions.

Since no universal heavy metal accumulators have been found among the studied plants, the exclusion of monophagous patterns in the diet of wild plants can be a reliable means of preventing metal toxicity in self-rescue situations on the seashore.

When assessing the suitability of natural plants from different habitats to serve as raw materials for the production of specialized food products, dietary supplements, and beverages enriched with essential elements, it is important to pay attention not only to the content of elements with micronutrient properties, but also to determining the full range of sorbed heavy metals.

The results obtained can be used when creating survival guides that recommend the consumption of wild plants.

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EXTENT OF PRE-HOSPITAL MEDICAL CARE TO CIVILIANS WITH ABDOMINAL GUNSHOT WOUNDS

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Introduction. Abdominal gunshot wounds account for 4.7–16.2% of injuries among their total number. Such wounds carry a high risk of fatal outcomes (depending on the nature of the wound, whether isolated or combined), as well as a large number of complications. In this regard, provision of proper and timely pre-hospital medical care is a highly important task.
Objective. To assess the extent and quality of pre-hospital medical care provided to civilians with gunshot abdominal wounds.
Materials and methods. The quality of emergency medical care was assessed based on a retrospective analysis of source documents: run sheets (Form 114/u), and records of 60 civilian patients (47 (78.3%) men and 13 (21.7%) women; average age 35 ± 5 years) in the special military operation (SMO) war zones. All injured were divided into two groups: (1) 46 (76.7%) wounded patients having received medical care from emergency medical teams (EMT) staffed with physicians and (2) 14 (23.3%) wounded patients having received medical care from EMTs staffed with paramedics. The EMT response time and extent of medical aid were assessed. The severity of the patient's state was assessed using the Battlefield Surgery Emergency Scale.
Results. It was found that the ambulance response time varied 5–30 min and averaged 24 ± 4 min for physician EMTs and 21 ± 6 min for paramedic EMTs, which can generally be described as normal. In total, 57 (85%) wounded had projectile wounds, with gunshot wounds being recorded in 3 (5%) cases. Multiple wounds were predominant in 52 (86.7%) cases, whereas single wounds were noted in 8 (13.3%) cases. A non-severe, severe, extremely severe, and critical state was recorded in 38 (63.3), 9 (15%), 12 (15%), and 1 (1.7%) patients. In the vast majority of cases (54 (90%)), the provided care was timely, proper, and to the full extent. At the same time, in 6 (10%) cases, the extent of provided emergency medical care could be considered insufficient: in 2 (3.3%) cases with physician EMTs and in 4 (6.7%) with paramedic EMTs. The errors were related to underestimating the severity of the patient' state, which resulted in inadequate anesthesia and infusion therapy, i.e., the absence of antishock actions.
Conclusion. Pre-hospital medical care to injured civilians with abdominal gunshot wounds is provided by physician and paramedic EMTs. The extent of medical aid includes wound treatment and aseptic dressing application, adequate anesthesia, and antishock actions. A lower error rate in the provision of emergency medical care by physician EMTs in comparison with paramedic EMTs was observed. Centralized measures should be implemented to improve both the theoretical knowledge and practical skills of EMTs in providing pre-hospital emergency medical care for abdominal gunshot wounds. To that end, it is necessary to involve surgeons and disaster medicine specialists in training emergency medical personnel.

Keywords: abdominal gunshot wounds; civilians; emergency medical care; complications; mortality

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ОБЪЕМ ОКАЗАННОЙ МЕДИЦИНСКОЙ ПОМОЩИ ГРАЖДАНСКОМУ НАСЕЛЕНИЮ ПРИ ПУЛЕВЫХ И ОСКОЛОЧНЫХ РАНЕНИЯХ ЖИВОТА НА ДОГОСПИТАЛЬНОМ ЭТАПЕ

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Введение. Огнестрельные ранения живота встречаются в 4,7–16,2% от общего числа ранений. Такие ранения сопровождаются высокими показателями летальных исходов (их количество зависит от характера ранений: изолированные или сочетанные), а также достаточно большим количеством осложнений. Исходя из этого, немаловажной задачей для снижения количества осложнений и летальных исходов является правильная и своевременная организация помощи на догоспитальном этапе.
Цель. Определить объем и правильность оказанной медицинской помощи на догоспитальном этапе пострадавшим с огнестрельными ранениями живота из числа гражданского населения.
Материалы и методы. Проведена оценка качества оказания неотложной медицинской помощи на основании ретроспективного анализа первичной документации: сопроводительных листов станций скорой помощи, талонов к ним (ф. 114/у) и историй болезни 60 пациентов (47 (78,3%) мужчин и 13 (21,7%) женщин; средний возраст 35 ± 5 лет) из числа гражданского населения в районах военных действий специальной военной операции (СВО). Все пострадавшие были разделены на две группы: в первую вошли раненые, которым помощь была оказана врачебными бригадами скорой медицинской помощи (СМП), — 46 (76,7%) человек, во вторую —

14 (23,3%) пациентов с оказанием помощи фельдшерскими бригадами СМП. Оценивали время доезда до пациента бригад скорой медицинской помощи, объем оказанной медицинской помощи. Оценка тяжести состояния проведена с использованием военно-полевой хирургической шкалы скорой помощи.

Результаты. Установлено, что время доезда бригад СМП варьировало от 5 до 30 мин и в среднем составило для врачебных бригад 24 ± 4 мин, для фельдшерских — 21 ± 6 мин, что в целом можно охарактеризовать как нормативное. У 57 (85%) раненых были отмечены осколочные ранения, огнестрельные ранения регистрировали в 3 (5%) случаях. У пострадавших преобладали множественные ранения в 52 (86,7%) случаях, тогда как одиночные ранения были отмечены в 8 (13,3%) наблюдениях. Нетяжелое состояние регистрировали у 38 (63,3) раненых, тяжелое — у 9 (15%), крайне тяжелое — у 12 (15%), критическое — у 1 (1,7%) пострадавшего. В подавляющем большинстве (54 (90%) наблюдения) оказанная помощь была своевременной, правильной и в полном объеме. Одновременно с этим в 6 (10%) наблюдениях объем выполненной скорой медицинской помощи можно считать недостаточным: в 2 (3,3%) наблюдениях при оказании помощи врачебными бригадами и в 4 (6,7%) — при оказании помощи фельдшерскими бригадами. Ошибки были связаны с недооценкой тяжести состояния пострадавших, как следствие — отсутствием выполнения адекватного обезболивания и проведения инфузионной терапии, т.е. непроведением противошоковых мероприятий.

Заключение. При огнестрельных ранениях живота пострадавшим из числа гражданского населения на догоспитальном этапе медицинская помощь оказывается врачебными и фельдшерскими бригадами скорой медицинской помощи. Объем помощи заключается в обработке раны и наложении асептической повязки, адекватном обезболивании и проведении противошоковых мероприятий. Отмечен более низкий процент ошибок при оказании скорой медицинской помощи врачебными бригадами СМП по сравнению с помощью, оказанной фельдшерскими бригадами СМП. Необходимо централизованное внедрение мероприятий по улучшению как теоретических знаний оказания скорой медицинской помощи при огнестрельных ранениях живота на догоспитальном этапе, так и отработки практических навыков бригадами СМП. С этой целью для обучения персонала СМП необходимо привлекать врачей-хирургов и специалистов по медицине катастроф.

Ключевые слова: огнестрельные ранения живота; гражданское население; скорая медицинская помощь; осложнения; летальность

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INTRODUCTION

Abdominal gunshot wounds occur in 4.7–16.2% of injuries among their total number [1]. Such injuries carry a high risk of fatal outcomes, which vary 8–36% for isolated wounds and 39.7–80% for combined wounds. Moreover, these wounds lead to a large number of complications in the postoperative period (approximately 36–65%) [2].

In this regard, provision of high-quality and timely pre-hospital medical care can significantly reduce the number of complications and fatalities. As a rule, such injuries are accompanied by shock and blood loss, which require competent pre-hospital medical care [3, 4]. This problem is acquiring particular importance due to the ongoing military actions; however, despite the current attention, many issues remain unresolved, such as delays in providing first aid [5] and a lack of coordination between agencies [6].

This study is aimed at determining the extent and quality of pre-hospital medical aid provided to civilians with abdominal gunshot wounds.

MATERIALS AND METHODS

The quality of emergency medical care was assessed based on a retrospective analysis of source documents: run sheets (Form 114/u) and records of 60 civilian patients (47 (78.3%) men and 13 (21.7%) women; average age 35 ± 5 years) who had received a gunshot abdominal wound in the special military operation (SMO) war zones.

The inclusion criteria for the study were as follows: a gunshot abdominal wound; the victim's age of at least 18 years old; and reception of emergency medical care from medical professionals. The exclusion criteria were as follows: patients with multiple wounds in other anatomical areas; patients younger than 18 years old; and patients having received pre-hospital medical care from non-medical professionals.

All the wounded were divided into two groups: (1) 46 (76.7%) wounded patients treated by emergency medical teams (EMTs) staffed with physicians and (2) 14 (23.3%) patients treated by EMTs staffed with paramedics. The EMT response time, extent of provided medical aid in accordance with the Order of

Ministry of Public Health[1], medical aid quality and timeliness were assessed. The severity of the patient's state was assessed using the Battlefield Surgery Emergency Scale: less than 20 points indicated a non-severe state at admission, 20–31 points indicated a severe state, 32–45 points indicated an extremely severe state, and more than 45 points indicated a critical state [7].

All primary data on each surveyed person was entered into an electronic database — a card index of Excel format tables. Data processing was carried out using the Microsoft Excel software package.

RESULTS

The study found that the EMT response time ranged 5–30 min, with an average of 24 ± 4 min for physician EMTs and 21 ± 6 min for paramedic EMTs, which can be considered a normal value. This time depended on such factors as the time of day (increasing during the day and decreasing at night), the overall situation in the locality (presence of debris, etc.), and the distance from the ambulance station or other medical facilities to the incident site.

In the study, the vast majority of cases, i.e. 57 (85%) wounded had projectile wounds, while gunshot wounds were recorded in 3 (5%) cases. In addition, multiple wounds were prevalent in 52 (86.7%) of the cases, while single wounds were observed in 8 (13.3%) cases.

The assessment of the patients' state using the Battlefield Surgery Emergency Scale in two groups detected a non-severe state in 30 (50%) and 8 (13.3%) patients in groups 1 and 2, respectively. A severe state was observed in 7 (11.7%) and 2 (3.3%) cases, respectively. An extremely severe state was recorded in 8 (13.3%) and 4 (6.7%) cases, respectively. One critical case (1.7%) was observed in group 1. The state severity was primarily due to shock, which was observed in 26 (43.3%) cases: in 22 (36.7%) cases in group 1, and in 4 (6.7%) cases in group 2.

The triage according to the class of hemorrhagic shock showed that 2 (3.3%) patients from group 1 and 3 (5.0%) patients from group 2 had Class I hemorrhagic shock; 18 (30%) and 1 (1.7%) patient, respectively, had Class II hemorrhagic shock, and 6 (10%) patients from group 1 had Class III hemorrhagic shock.

It should be noted that in 12 (60%) of the cases, signs of a penetrating abdominal injury were detected during the examination of the wounded, which manifested itself in the pathologic discharge from the wound. These signs were observed in 9 (15%) of the wounded who were treated by physician EMTs, and in 3 (5%) of the wounded who were treated by paramedic EMTs.

The types of assistance provided by physician and paramedic teams to injured patients at the scene are listed in Table 1.

Table 1 shows that the measures of wound debridement and aseptic dressing application were performed to the full extent, both by physician and paramedic EMTs. However, in 6 (10%) cases, the extent of emergency medical aid was considered insufficient: in 2 (3.3%) cases provided by physician EMTs and in 4 (6.7%) cases provided by paramedic EMTs. The errors were related to underestimating of the patient's state severity, which resulted in inadequate anesthesia and infusion therapy, i.e., the failure of antishock actions. In other cases, infusion therapy was fully implemented throughout the period of evacuation of the injured patient to a medical facility.

An analysis of the immediate postsurgical period showed that complications developed in 15 (25%) of the total number of wounded patients. The nature and number of complications recorded in patients of both groups are presented in Table 2.

It is noteworthy that all the complications were related to wound contamination, and the group of patients who received pre-hospital care from paramedic EMTs was more likely to develop such complications. This may be due to the higher percentage of reported errors in this group.

Table 1. Types of medical care provided by ambulance teams at the site of abdominal gunshot wounds

EMT	Casualty load, *n*	Types of medical care			
		Wound debridement	Wound dressing	Anaesthesia	Infusion therapy
Physician	46	46 (76.7%)	46 (76.7%)	44 (73.3%)	44 (73.3%)
Paramedic	14	14 (23.3%)	14 (23.3%)	10 (16.7%)	10 (16.7%)

Table prepared by the authors using their own data

[1] Order of Ministry of Public Health of the Russian Federation dated 20 June 2013. No. 388n "On Approval of the Procedure for Providing Emergency Medical Care, Including Specialized Emergency Medical Care".

Table 2. Nature and number of complications in abdominal gunshot wounds in the immediate postsurgical period

EMT	Casualty load, *n*	Complication type			
		Postoperative wound infection	Peritonitis	Pneumonia	Abdominal infiltrate
Physician	46	7 (11.7%)	3 (5%)	11 (18.3%)	8 (13.3%)
Paramedic	14	6 (10%)	4 (6.7%)	4 (6.7%)	3 (5%)

Table prepared by the authors using their own data

The total mortality in both groups was 10 (16.7%) cases. Among patients who received medical care from physician EMTs, the mortality rate was lower — 4 (6.7%) cases, while among patients who received medical care from paramedic EMTs, the mortality rate was 6 (10%). However, among patients who received medical care from paramedic EMTs, 4 (6.7%) patients died during transportation to a medical facility. The main cause of death among the wounded was shock in 8 (13.3%) cases: 2 (3.3%) in group 1 and 6 (10%) in group 2. The remaining 2 (3.3%) patients in group 1 died due to diffuse peritonitis caused by multiple injuries to the hollow organs of the abdominal cavity.

DISCUSSION

The conducted study shows that issues related to the timely and proper organization of pre-hospital emergency medical care for civilians in cases of abdominal gunshot wounds are of great importance. According to Smelaya et al. [9], an improved medical evacuation system for modern combat trauma can lead to better treatment outcomes and mortality decrease. The literature pays significant attention to the challenges of providing pre-hospital care [10]. At the same time, the main factors affecting the treatment outcomes for such injuries include time from the injury to the reception of medical care, as well as the appropriateness of the measures taken. Both physician and paramedic EMTs can provide pre-hospital emergency medical care. This issue is particularly relevant today, in the context of high risks of terrorist attacks and military operations on the territory of the Russian Federation.

According to the results obtained, the ambulance response time did not exceed the established limit, but depended on various factors. The quality of emergency medical care is an important factor that affects the postsurgical period in cases of gunshot wounds. As a rule, abdominal wounds are accompanied by the development of shock, which is caused by both pain and acute blood loss.

Another factor that can worsen the patient's state is infection. Therefore, the high-priority task of emergency medical care is wound debridement and aseptic dressing application to prevent contamination. The analysis showed that all EMT professionals successfully completed this task, regardless of whether they were paramedic or physician teams. However, the early antibacterial prophylaxis for gunshot abdominal wounds remains a controversial issue. The main errors identified during the analysis were underestimation of the patients' severity and insufficient antishock actions. Moreover, more errors were observed when paramedic teams provided medical care.

The data obtained can be used to formulate practical guidelines aimed at improving both the theoretical knowledge and practical skills of emergency medical teams regarding pre-hospital emergency medical care in the case of abdominal gunshot wounds. To that end, it is necessary to involve surgeons and disaster medicine specialists in training emergency medical personnel.

It is evident that the course of the immediate postsurgical period depends on various factors, which might be ignored at the pre-hospital phase. However, timely and proper treatment of such injuries can reduce the number of complications and fatal outcomes.

CONCLUSION

Pre-hospital medical care was provided by physician and paramedic emergency medical teams to civilian victims with abdominal gunshot wounds. The extent of medical aid included wound debridement and aseptic dressing application, adequate anesthesia,

and antishock actions. In the vast majority of cases, medical care was timely, proper, and to the full extent. There was a lower percentage of errors in the extent of medical aid provided by teams staffed with physicians compared to those staffed with paramedics. The errors were related to underestimation of the patients' state severity and, as a result, insufficient antishock actions.

The total mortality was lower among patients treated by physician teams, and the rate of uncomplicated postsurgical periods was also higher among these patients. The complications were mostly related to wound contamination, and the complication incidence was higher among patients treated by paramedic teams.

In order to improve the efficiency and quality of emergency medical care, emergency medical personnel should undergo training sessions on issues related to providing medical care to patients with abdominal gunshot wounds, with the involvement of specialists in battlefield surgery and disaster medicine. In our opinion, such training sessions should be organized at disaster medicine departments, which typically have significant experience in these areas.

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APPLICATION PROSPECTS OF PLANT AND FUNGAL COMPOUNDS IN ANTITUMOR THERAPY

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Introduction. Anticancer inhibitors of plant and fungal origin (IPFOs) represent a promising direction in antitumor therapy, offering a variety of mechanisms of action, in most cases different from conventional chemotherapeutic drugs. As a rule, IPFOs simultaneously affect several metabolic pathways, exerting a combined effect on different targets in the cancer cell and reducing the risk of drug resistance development.
Objective. To study promising directions in the development of new antitumor drugs, to generalize current data on the IPFO mechanism of action in the context of a combined approach to cancer treatment.
Discussion. Compounds exhibiting antitumor activity are increasingly attracting the research attention. Due to their diverse mechanisms of action, anticancer IPFOs represent a promising direction in cancer treatment. A large number of conventional chemotherapy drugs, although being of plant origin, demonstrate high effectiveness, which confirms the relevance of searching for new anticancer IPFO compounds. Solid tumors exhibit a pronounced ability to both proliferate and induce angiogenesis, which justifies the current active search for new plant-derived compounds with antiangiogenic properties, along with other IPFOs. As a rule, anticancer IPFOs simultaneously affect several metabolic pathways, exerting a combined effect on different targets in the cancer cell and reducing the risk of drug resistance.
Conclusions. This review has examined the molecular mechanisms of IPFO action, including suppression of angiogenesis and cancer cells proliferation, apoptosis induction, cell cycle modulation, and direct cytotoxic effect by stimulating the activity of $CD8^+$ T lymphocytes, NK cells, and macrophages.

Keywords: antitumor therapy; plant- and fungus-derived tumor inhibitors; apoptosis; programmed cell death; angiogenesis; autophagy; ferroptosis; cell cycle regulation

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ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ СОЕДИНЕНИЙ РАСТИТЕЛЬНОГО И ГРИБНОГО ПРОИСХОЖДЕНИЯ В ПРОТИВООПУХОЛЕВОЙ ТЕРАПИИ

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Введение. Ингибиторы растительного и грибного происхождения (ИРГП) представляют собой перспективное направление в противоопухолевой терапии, предлагая разнообразные механизмы действия, в большинстве случаев отличающиеся от традиционных химиотерапевтических препаратов. Как правило, ИРГП одновременно влияют на несколько метаболических путей, что снижает вероятность развития резистентности, оказывая комбинированный эффект на разные мишени в раковой клетке.
Цель. Изучить перспективные направления в создании новых противоопухолевых препаратов для последующего лечения, обобщить современные данные о механизмах действия ИРГП в контексте комплексного подхода к лечению злокачественных опухолей.
Обсуждение. В настоящее время усиленно проводится поиск новых соединений с противоопухолевым потенциалом. ИРГП представляют собой перспективное направление в противоопухолевой терапии, предлагая разнообразные механизмы действия. Многие традиционные химиотерапевтические препараты также имеют растительное происхождение и обладают хорошей эффективностью, что подтверждает актуальность изучения данной тематики. Солидные опухоли обладают повышенной способностью к активной пролиферации и ангиогенезу, что объясняет неизменный интерес к активному поиску новых соединений растительного происхождения с антиангиогенными свойствами, наряду с исследованиями других ИРГП. Как правило, ИРГП одновременно влияют на несколько метаболических путей, что снижает вероятность развития резистентности, оказывая комбинированный эффект на разные мишени в раковой клетке.
Выводы. В обзоре рассмотрены молекулярные механизмы действия ИРГП, включающие в себя подавление ангиогенеза и пролиферации раковых клеток, индукцию апоптоза, модуляцию клеточного цикла, а также прямой цитотоксический эффект путем стимуляции активности $CD8^+$ Т-лимфоцитов, NK-клеток и макрофагов.

Ключевые слова: противоопухолевая терапия; растительные и грибные ингибиторы опухолей; апоптоз; запрограммированная клеточная смерть; ангиогенез; аутофагия; ферроптоз; регуляция клеточного цикла

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INTRODUCTION

Although a wide range of effective antitumor drugs have been developed to date, this field continues to remain relevant due to the search for optimal drug combinations with the least number of side effects. In this connection, plant- and fungus-derived compounds are attracting particular attention [1]. The available data suggest that natural compounds affecting autophagic and apoptotic pathways are effective mediators of cancer therapy with specificity for target cancer cells. The multidirectional antitumor effect of natural compounds in combination with their low toxicity are significant prerequisites for the development of drugs for cancer prevention and cancer treatment [1].

Cancer inhibitors of plant and fungal origin (IPFOs) have slightly different mechanisms of action in antitumor therapy. The plant-derived cancer inhibitors mainly affect the cellular signaling pathways of carcinogenesis, while exhibiting an anti-inflammatory effect. The fungus-derived cancer inhibitors are capable of stimulating the immune response with subsequent tumor recognition or preventing cancer cell division. An important advantage of IPFOs as candidate substances consists in the presence of properties essential for all drug medications, such as gastrointestinal absorption and metabolism action. In addition, IPFOs demonstrate a high chemical diversity necessary to study correlations between activity and structure [2]. Some first plant-derived drugs (paclitaxel, vinblastine, vincristine, topotecan, irinotecan, and teniposide), have been extensively studied, partially modified, and approved by the U.S. Food and Drug Administration (FDA, USA). These medicines are not the subject of this review [3].

In this study, we analyze promising directions in the development of anticancer drugs, review the current data on the mechanisms of IPFO action in the context of a combined approach to cancer treatment.

MATERIALS AND METHODS

In addition to the Google Scholar and PubMed search system, the Naturally Occurring Plant-based Anti-cancerous Compound-Activity-Target Database (NPACT, http://crdd.osdd.net/raghava/npact/) was used. NPACT features about 1980 experimentally confirmed interactions of compounds and targets. The search queries included the following keywords: anticancer therapy; plant and fungal cancer inhibitors; apoptosis; programmed cell death; angiogenesis; autophagy; ferroptosis; cell cycle regulation (in Russian and English). The search depth was 10 years. The inclusion criteria were the relevance and practical significance of the publications, as well as the availability of preclinical and clinical trial data.

RESULTS AND DISCUSSION

The search for compounds with antitumor potential is currently underway. Anticancer IPFOs represent a promising direction in cancer treatment, offering a variety of mechanisms of action. There is a large number of conventional chemotherapy drugs with high effectiveness, which are also of plant origin. This circumstance justifies the relevance of searching for new compounds. Solid tumors exhibit a pronounced ability to both proliferate and induce angiogenesis, driving the need to develop new plant-derived compounds with antiangiogenic properties, along with the investigation of other IPFOs. As a rule, anticancer IPFOs affect several metabolic pathways simultaneously, exerting a combined effect on different targets in the cancer cell and reducing the risk of drug resistance development.

Among the main methods used to identify compounds with a certain activity are gene cloning, DNA and RNA sequencing, studying the compounds effect on the enzymes activity involved in the relevant metabolic pathways, evaluating differential gene expression using microarrays, flow cytometry, the use of various cell cultures, including those of tumor origin, the use of animal models to assess the systemic effect of the compound, as well as its pharmacokinetics and pharmacodynamics, multidimensional statistical analysis to assess the reliability of the results obtained. The largest amount of information on natural anticancer compounds in NPACT concerns those derived from plants [4]. Many anticancer IPFO have been isolated from herbs used in traditional Chinese medicine [5]. However, it should be noted that not only plant-derived, but also fungus-derived, medications have been found to possess anticancer potential. The key classes of inhibitory compounds for plants and fungi are phenolic compounds and terpenoids. At the same time, in plants, it is alkaloids, flavonoids, and coumarins that demonstrate similar anticancer properties, while in fungi, these are polysaccharides, glucans, steroids, cerebrosides, and proteins. The diverse mechanisms of action of such compounds include apoptosis induction, inhibition of angiogenesis and the cell cycle, immunomodulation, reprogramming of cellular signaling pathways involved in carcinogenesis, as well as various antioxidant and anti-inflammatory effects (Table 1).

Induction of programmed cell death

Apoptosis

Apoptosis, i.e., programmed cell death, occupies a special place among various processes associated with cell cycle regulation, correct functioning of the immune system, hormone-dependent atrophy, and embryo development [9]. The ability of chemical compounds to induce apoptosis determines their significant

Table 1. Classes of inhibitor compounds and their mechanisms of action

Plants	Fungi
Inhibitory compound classes	
alkaloids	polysaccharides
phenolic compounds (polyphenols)	
flavonoids	glucans
terpenoids	
coumarins	steroids
	cerebrosides
	proteins
Mechanisms of action	
induction of cancer cell apoptosis (programmed cell death)	
angiogenesis inhibition (formation of new blood vessels feeding the tumor)	
Modulation of cellular signaling pathways involved in cancer development	Immunomodulation: Some fungus-derived compounds can stimulate the immune system to recognize and attack tumor cells. This includes increased activity of natural killer cells (NK), T lymphocytes, and macrophages
Antioxidant and anti-inflammatory effects	Cell cycle inhibition: other drugs affect the most important proteins and processes involved in cytokinesis, preventing tumor cell reproduction

Table prepared by the authors using data from the references [1–3, 5, 6–8]

therapeutic potential. The following IPFOs can serve as such examples.

Icaritin induces programmed cell death of ovarian cancer cells by activating the apoptosis pathway through p53 and inhibiting the Akt/mTOR signaling pathway [10]. The curcumin anticancer activity may directly depend on the effect on the p53 pathway in human osteosarcoma (HOS) cells [11]. The triggering of internal and external apoptosis pathways is also responsible for the curcumin anticancer effects in monocytic leukemia (SHI-1) cells [12]. It was shown that matrine is capable of stimulating the main apoptotic cascades by increasing the accumulation of Fas and FasL, Bax proteins, while reducing the amount of Bcl-2 apoptosis regulator, which leads to activation of caspases-3, -8, and -9 in human osteosarcoma MG-63 cells, as well as U-2OS, Saos-2, and MNNG/HOS [6, 13]. The molecular mechanism of action of tetrandrine in cancer cells is aimed at increasing the number of Bax, Bak, Bad, and Apaf-1 apoptotic proteins, while the number of Bcl-2 and Bcl-xl antiapoptotic proteins in the cell decreases with the release of cytochrome *c* (cyt *c*) and activation of caspase-3 and caspase-9 in the mitochondrial pathway of apoptosis [14, 15].

A number of studies on animal models showed that epigallocatechin gallate (EGCG) can inhibit the growth of malignant cells and induce apoptosis even in cancer cell lines resistant to CD95-mediated apoptosis [16]. Saikosaponin A has proapoptotic activity, namely, it positively regulates the pathway mediated by Bax/Bcl-2/caspase-9/caspase-7/PARP [17], causing apoptosis of human colon cancer cells SW480 and SW620 (colon cancer cell lines derived from primary tumors and lymph node metastases, respectively) in a dose-dependent manner, which is obviously related to the inhibition of the PI3K/Akt/mTOR signaling pathway [18]. Bavachinin can influence the expression of Bcl-2, Bax, caspase-3/9, and the peroxisome proliferator γ (PPARγ) receptor. The bavachinin-induced generation of reactive oxygen species (ROS) depends on the PPARγ activation, which is capable of inducing A549 cell death. The effect caused by an increase in the level of reactive oxygen species (ROS) highlights the potential role of bavachinin as a chemotherapeutic agent against non-small cell lung cancer [19]. Gossypol can interact with the BH3 domain binding groove of the Bcl-xL and Bcl-2 antiapoptotic proteins. Simultaneous incubation of non-Hodgkin's lymphoma Ramos cells with gossypol and etoposide enhances

apoptosis due to the intensive release of cytosolic cyt *c* and activation of caspase-3 signaling depending on time intervals. These results are the basis for future preclinical and clinical studies of gossypol in the treatment of non-Hodgkin's lymphoma [20]. It was shown that the action mechanism of resveratrol involves the blocking of certain transcription factors, such as B cell nuclear factor (NF-kB), AP-1 and Egr-1, as well as a decrease in the expression of antiapoptotic genes and activation of caspases. Its ability to influence the immune response mediated by B cells and increase the serum level of antibodies, exerting an antitumor effect, was previously revealed [21].

Autophagy and ferroptosis

Autophagy is a cellular degradation process leading to the removal of improperly folded or aggregated proteins, as well as the degradation of damaged organelles such as mitochondria, endoplasmic reticulum (EPR), and peroxisomes [22]. Autophagy can inhibit the growth and progression of malignant tumors, since the removal of damaged or non-functioning organelles prevents oncogenesis. At the same time, stimulation of autophagy remains an effective approach in antitumor therapy.

The mammalian target of rapamycin, mTOR, which has the properties of a modulator of cell growth and proliferation, and AMP-activated protein kinase (AMPK), responsible for signal conversion in response to various metabolic stresses, are regulators of autophagy initiation [23]. This process prevents tumor development only provided the possibility of selective autophagy, targeting certain cell organelles [24]. In cases where a tumor has already formed, autophagy suppression often leads to the development of less aggressive cancer forms [25]. It was shown that synthetic quinine analogues — chloroquine (CQ), isolated from the cinchona bark (*Cinchona officinalis*), and hydroxychloroquine (HCQ) — are the most common drugs used to treat acute and chronic inflammatory diseases. These drugs are also used in cancer treatment based on autophagy inhibition mechanisms, which consist in interrupting the fusion of autophagosomes and lysosomes [26].

According to Solomko et al., matrine-induced signals in tumor cells can lead to ferroptosis (has a protective effect against cervical cancer) [6, 13]. Matrine exhibits considerable antitumor activity both *in vitro* and *in vivo*, along with other beneficial properties, e.g., antianxiety and antidepressive effects, relieving neuroinflammation in the brain caused by severe diseases. The mechanism of action of this compound is based on the suppression of cell proliferation and apoptosis induction, e.g., the highly metastatic breast cancer cell line MDA-MB-231 uses the VEGF-Akt-NF-kB signaling pathway. Unfortunately, numerous anticancer drugs (such as etoposide, tyrosine kinase inhibitors, arsenic trioxide, 5-fluorouracil) that cause ferroptosis are cardiotoxic [27]. To address this issue, non-toxic cardioprotective antitumor plant-derived medications with anticancer activity, such as berberine, epigallocatechin gallate, and resveratrol, have been developed and are used in combination with conventional chemotherapeutic agents [16, 21, 28].

Angiogenesis inhibition

Tumors induce the growth of new blood vessels due to releasing various growth factors, including vascular endothelial growth factor (VEGF). This results in the formation of blood capillaries inside the tumor. Protein Kinase G (PKG) regulates beta-catenin levels in healthy cells, promoting angiogenesis. Angiogenesis, in turn, is an important factor in the spread of tumor metastases. Fennel extracts — *Trianthema portulacastrum* and *Spatholobus suberectus* — inhibit tumor growth and angiogenesis, as well as alter the expression of HSP90 heat shock protein and its co-chaperone interactions in mouse models of breast cancer. These findings on the role of HSP90 in breast cancer biology and therapy are consistent with the effects described in the current literature. In fact, tumor growth and angiogenesis decrease when HSP90 is suppressed by the interaction of the KU-32 inhibitor with the C-terminal domain of this chaperone in trastuzumab-resistant HER2-positive breast cancer cells [29]. Morelloflavone blocks injury-induced neointimal hyperplasia by inhibiting vascular smooth muscle cell migration without causing apoptosis or cell cycle arrest [30]. Thus, the use of certain compounds of natural origin suppresses the formation of new blood vessels that require a significant amount of oxygen and nutrients for their growth, which can increase the antitumor effect [7].

Modulation of cellular signaling pathways

MAPK paths. Phytochemicals can affect both the cascade pathway kinase regulated by extracellular signals (ERK) and mitogen-activated protein kinases (MAPK) regulating cell growth and cell survival. Phytochemicals such as ursolic acid, kaempferol, resveratrol, gingerol, sulforaphane, genistein, and isothiocyanates have been reported to induce cancer cell apoptosis through the MAPK and ERK pathways [31]. It was shown that curcumin exerts the anticancer effect on retinoblastoma cells (RB, Y79) by activating exclusively the MAPK pathway.

Akt signaling pathways. The Act/PI3 signaling pathway plays an important role in cancer development. Epidermal growth factor (EGF) regulates a number of molecular mechanisms, including NF-kB activation and Akt phosphorylation. This promotes resistance to apoptosis and uncontrolled cell proliferation, which in turn leads to effects on caspases, Bcl-2 and glycogen synthase 3-β (GSK3b) kinases, as well as mTOR. Alkaloids and phenolic compounds make a significant contribution to the control over the expression of these factors. Resveratrol, curcumin, luteolin, flavone, and sulforaphane

exhibit anticancer properties by ceasing the cell cycle and apoptosis, interfering with Akt/PI3K signaling [32]. In addition, Saikosaponin A inhibits the invasion and migration of SK-N-AS cells (human neuroblastoma cells) by regulating the angiogenesis-associated VEGFR2/Src/Akt pathway and protein expression associated with epithelial-mesenchymal transition (EMT) [17].

JAK/STAT signal transmission paths. By inhibiting the activity of JAK/STAT signaling and activating apoptotic cascades, the curcumin, resveratrol, and EGCG phytochemical compounds inhibit the translocation and collection of β-catenin in the nucleus by stimulating glycogen synthase kinase 3 (GSK3), which can lead to cell death in some forms of cancer [33].

Antioxidants with anti-inflammatory effects

Harmala extract (*Peganum harmala*) can reduce the viability of cervical carcinoma and colon cancer cells due to the action of alkaloids contained in high concentrations therein. In a study aimed at investigating cytotoxicity towards normal and tumor cells, the antioxidant activity of these alkaloids against human breast cancer cells was noted [34]. Three main epigenetic changes occur in tumor cells treated with plant polyphenols, i.e., a change in the structure of chromatin, DNA methylation, and, more importantly, alterations in the microRNAs level. For the same microRNAs, expression is increased in some tumors, while in others, on the contrary, it is reduced. Thus, the expression of the mi-Let-7 cluster increases in breast tumors and, conversely, decreases in lung tumors. Notably, EGCG, curcumin, and resveratrol modulate several classes of microRNAs that are involved in all stages of cancer development and regulate oncogenes or tumor suppressors of various cancers [16, 21]. In particular, tetrandrine was shown to exhibit antiproliferative effects and cytotoxic activity against breast cancer (MDA-MB-231, HCC1937, MCF7) [14, 15].

Cell cycle regulation

The cell cycle is a sequence of intracellular events, leading to cell division. The stages of the cell cycle are mediated by cyclin-dependent kinases (CDK) and their regulatory cyclin subunits [35]. Vindoline and catharanthine have antitumor effects due to their exposure in the M-phase cell cycle. They contribute to the cancer cell death by shortening microtubules and disrupting their function, which leads to the disappearance of mitotic spindle, thereby suppressing cell proliferation [34].

Quercetin can affect the cell cycle at the G1/S and G2/M control points by inducing the CDK p21 inhibitor and reducing the phosphorylation of the key regulatory pRb protein and indirectly blocking E2F, which are important factors of transcription and DNA synthesis [36]. The roscovitin synthetic compound, produced from the natural substance olomucine isolated from the *Raphanus sativus* (*Brassicaceae*) daikon, has passed clinical trials, showing high activity against various types of cancer. This medication is currently at the stage of clinical evaluation of efficacy in the treatment of Cushing's disease and rheumatoid arthritis [37]. This drug is an inhibitor of cyclin-dependent kinases, preventing their activation and DNA repair due to non-homologous end joining (NHEJ). One of the most noticeable effects of the drug is the inhibition of formation of CDK2/cyclin E complexes, which causes a decrease in the pRb phosphorylation level and subsequent inactivation of members of the E2F family, leading to suppression of cyclin transcription and, ultimately, to cell cycle arrest. In this case, cell cycle arrest leads to the initiation of apoptotic death [6]. The modes of flavopiridol action are associated with the phosphorylation of cyclin-dependent kinases that block cell proliferation in G1 and G2 phases, and the apoptosis induction by increasing the level of E2F synthesis and Mcl-1 protein inactivation. A study in which the effect of EGCG on oncogenesis was tested on oral cancer (NOE) cell lines together with curcumin showed the EGCG ability to block cell division in G1, whereas curcumin blocked cell division in S/G2/M phases. The antagonistic interaction between curcumin and etoposide is caused by cell cycle arrest, which gives time for DNA damage to repair and prevents cell death. Another polyphenol, quercetin, may limit the effect of etoposide. Quercetin has a protective effect on HL-60 cells from etoposide, reducing the level of ROS generated in drug-treated cells (Table 2) [38, 39].

Fungus-derived compounds

Cancer fungotherapy and the search for new anticancer agents are not limited to such fungi species as *Fomitopsis pinicola*, *Hericium erinaceus*, *Trametes versicolor*, and *Inonotus obliquus* from the *Basidiomycota* class. However, these four species can serve as typical representatives of medicinal fungi, which are widely used both in conventional medicine and in modern biomedical research. They belong to three different groups and are a rich source of bioactive compounds such as polyphenols, polysaccharides, glucans, terpenoids, steroids, cerebrosides, and proteins that show potential for treating various types of cancer [8]. Ergosterol is an active *Fomitopsis pinicola* fungus-derived compound, which is the main component affecting SW-480 cells and causing their apoptosis. Interestingly, the use of this extract in combination with cisplatin, a common chemotherapeutic agent, in mice showed their synergistic effect of impeding tumor growth. Taken together, these results provide solid evidence that, in addition to nonspecific cytotoxic compounds, *F. pinicola* contains substances with a specific antioncogenic potential, probably acting through the induction of apoptosis [48].

Krestin, isolated from the mycelium of the *Trametes versicolor* wood fungus, belongs to the class of polysaccharides. This compound shows significant

Table 2. Antitumor inhibitors of plant and fungal origin

Title	Compound class	Extracted from	Mechanism	Cell lines	Literature source
Compounds of plant origin					
Vindoline and catharanthine	Alkaloid	*Vinca rosea*	Effect on the cell in the M-phase of cell cycle; shortening of microtubules, disruption of their function, which leads to the mitotic spindle disappearance, thereby suppressing cell proliferation	Kaposi's sarcoma, melanoma, nasopharyngeal cancer, breast cancer, kidney, bladder, breast cells, prostate, cervix (MCF-7, PC3-1C, HeLa)	[6]; [34]
Matrine	Alkaloid	*Sophora flavescens*	Stimulation of the main apoptotic cascades by accumulating Fas/FasL, Fas and reducing Bcl-2 levels, which leads to activation of caspase-3, -8 and -9	Human osteosarcoma cells (MG-63, U2OS, Saos-2 and NG/HAS)	[6]; [13]
Tetrandrine	Alkaloid	*Stephania tetrandra*	Positive regulation of the Bax, Bak, Bad, and apaf-1 pathways; reduction of Bcl-2 and Bcl-xl levels; release of cytochrome *c* and activation of caspase-3 and -9	Breast cancer cells (MDA-MB-231, HCC1937, MCF7)	[14]; [15]
Epigallocatechin gallate	Polyphenol	Green Tea, *Camellia sinensis*	Induction of apoptosis, arrest of cell growth by changing the expression of regulatory proteins of the cell cycle; activation of killer caspases and suppression of NFkB activation; inhibition of Bcl-2 and Bcl-XL expression, as well as induction of Bax, Bak, Bcl-XS and PUMA expression	*In vitro* model: esophagus; oral cavity; prostate; mammary gland; urinary tract; lungs; colon; leukemia; lymphoma. *In vivo* model: cancer of the skin, prostate, colon and uterus; cancer of the stomach, pancreas and oral cavity in humans	[16]; [21]
Curcumin	Polyphenol	Rhizome, *Curcuma longa*	Effects on the p53 pathway, activation of the MAPK pathway, an increase in the Bax:Bcl-2 ratio and the release of cytochrome *c*, the second mitochondrial activator of caspases/direct binding protein IAP	Human osteosarcoma cells (HAS), retinoblastoma cells (RB Y79), monocytic leukemia cells (SHI-1)	[11]; [12]; [38]; [40]
Resveratrol	Polyphenol	A component of white hellebore roots, *Veratrum grandiflorum*	Blocking of certain transcription factors such as NF kB, AP-1, and Egr-1; decreased expression of antiapoptotic genes and activation of caspases	Squamous cell carcinoma of the human esophagus	[6]; [21]; [31]; [32]
Gossypol	Polyphenolic aldehyde	Cotton plant, *Gossypium sp., Malvaceae*	Binding of antiapoptotic proteins Bcl-xL and Bcl-2 to the groove of the BH3 domain; enhancement of apoptosis due to the release of cytosolic cytochrome *c* and activation of caspase-3 signaling	Non-Hodgkin's lymphoma cells	[20]
Saikosaponin a	Terpenoid	*Radix Bupleuri,* root	Activation of the Bax/Bcl 2 cascade and caspase-9, caspase-3, associated with inhibition of the PI3K/Akt/mTOR signaling pathway	Human neuroblastoma cells (SK-N-AS), Human colon cancer cells (SW480, SW60)	[17]; [18]

Table 2 (continued)

Title	Compound class	Extracted from	Mechanism	Cell lines	Literature source
Bavachinin	Flavonoid	*Psoralea corylifolia,* legume family	PPARγ activation leading to ROS generation	Non-small cell lung cancer (A549)	[19]
Icaritin	Flavonoid	Traditional Chinese herb, *Epimedium Genus*	Activation of apoptosis via p53 and inhibition of the Akt/mTOR pathway	Ovarian cancer, leukemia, lymphoma and multiple myeloma	[10]; [41]; [42];
Quercetin	Flavonoid	Larch, *Larix*	Generation of free radicals that lead to oxidative damage of nucleic acids, lipid peroxidation and cell death; possibility to cause apoptosis through the AMPK-α or COX-2 signaling pathway	Human hepatocyte cells, epithelial cell lines, prostate cancer	[14]; [36]; [38]; [39]
Morelloflavone	Flavonoid	Seeds, *Garcinia morella*	Inhibition of activation of RhoA and Rac1 GTPases with insignificant effect on Cdc42 GTPase activation. Inhibition of phosphorylation and kinase activation of the Raf/MEK/ERK pathway, without affecting VEGFR2 activity	U87 glioma cells and C6 rat glioma cells	[30]; [43]; [44]
Fungus-derived compounds					
Krestin	Polysaccharide	Mycelium of wood, *Trametes versicolor*	The ligand for TLR4 receptors leads to the induction of inflammatory cytokines TNF-alpha and IL-6	Allogeneic and syngeneic tumors of animals	[45]; [46]
Lentinan	Polysaccharide	Wood fungus, *Lentinus edodes*	Stimulation of T-lymphocytes, induction of interleukins 1 and 3, as well as the production of nitric oxide by immune cells, leading to an increase in the level of colony-stimulating factor and the level of proteins of the acute phase of inflammation	Tumors of the stomach, bones and breast	[47]
Ergosterol	Polysaccharide	*Fomitopsis pinicola*	Induction of apoptosis: increased levels of pro-apoptotic proteins such as BAX, caspase-7 and PARP, and decreased amounts of anti-apoptotic proteins BCL-2 and STAT-3	Breast cancer cell lines	[48]; [49]
Aqueous extract		*Hericium erinaceus*	Suppression of antiapoptotic proteins (Bcl-2, Bcl-xL(S), XIAP and cIAPs) in the absence of increased proapoptotic proteins	Various cancer cell lines and tumors associated with the digestive tract	[50]; [51];

Table prepared by the authors using data from the references [6, 10–21, 46–49, 31, 32, 34, 36, 38–50]

antitumor activity against allogeneic and syngeneic animal tumors [45].

Polysaccharide-K (PSK) demonstrates a similar activity in various types of cancer, especially in gastrointestinal cancer. This medicine has been approved in Japan and China for use in cancer treatment [49]. Lentinan (isolated from *Lentinus edodes*) also belongs to the class of polysaccharides. This compound prevents neoplastic transformations caused by chemical carcinogens and viruses, while also suppressing the development of allogeneic and some syngeneic tumors. This polysaccharide is most often used in the treatment of tumors of the stomach, bones, and breast. The action mechanism of lentinan involves stimulation of $CD8^+$ T lymphocytes, induction of interleukins 1 and 3, as well as the production of nitric oxide by immune cells. This leads to an increase in the synthesis of colony-stimulating factor (CSF) and the accumulation of proteins of the inflammation acute phase in combination with direct and indirect (through T lymphocytes) effects on macrophages. Lentinan showed clinical efficacy in various types of cancer, including stomach and lung cancer [47]. Another study by the same scientific group was devoted to the use of conk extracts from the *Hericium erinaceus* fungus with various solvents and testing for cytotoxicity against human monocytic leukemia U937 cells. The results showed that both aqueous and ethyl extracts can induce apoptosis. The suggested mechanism of action is through the suppression of anti-apoptotic factors (Bcl-2, Bcl-xL(S), XIAP and cIAPs) in combination with the absence of an increase in the level of pro-apoptotic proteins.

Erinacin A, a mycelial derivative of *H. erinaceus*, demonstrates activity that suppresses the growth of various gastrointestinal tumor lines [50]. Extracts of *H. erinaceus* or their fractions/components were shown to exhibit immunostimulating activity; antimetastatic activity by inhibiting matrix metalloproteinases; activity promoting the growth of probiotic intestinal flora; antioxidant potential; proapoptotic activity; and angiogenesis inhibition. This range of anticancer properties is provided by various compounds, including polysaccharides, lipids, terpenoids (unique erinacins), and even proteins. Thus, there are two possible strategies for the investigation of *H. erinaceus* anticancer effects, such as studying the complex effects of extracts with their further use as preventive dietary supplements and a detailed study of the mechanisms of individual fungus-derived compounds for use in targeted personalized anticancer therapy [51]. The anticancer potential of the *Inonotus obliquus* fungus is represented by several groups of components. Unique triterpenoids such as lanostane, inotodiol, and inonotsuoxides act *in vivo* on preparations of mouse skin and tumors of mouse xenografts derived from human chronic lymphocytic leukemia. Low molecular weight polyphenolic compounds of this fungus can inhibit topoisomerase II, which leads to a decrease in the growth of cultured HCT116 human colon carcinoma cells. Similar to *H. erinaceus*, cinder conk (*Inonotus obliquus*) is extremely rich in polysaccharides, which can perform immunomodulatory functions and inhibit oncogenesis [52].

The *Cordyceps militaris* fungus showed promising results in preclinical studies of antiproliferative and antimetastatic effects against various types of cancer. *Ganoderma lucidum*, known as reishi, contains ganoderic acids, which have antitumor and immunomodulatory properties. *Grifola frondosa*, also known as maitake, contains such compounds as maitake polysaccharides, which demonstrated anticancer activity in preclinical studies [53].

CONCLUSION

Plants contain a diverse range of biologically active compounds, including alkaloids, flavonoids, terpenoids, and polyphenols, which have been traditionally used in medical practice. Despite promising laboratory results in suppressing tumor growth and metastasis, many plant compounds require further study and clinical trials to confirm their effectiveness and safety. Among the anticancer IPFOs analyzed in this review, which are not a substitute for conventional drugs but are promising for concomitant therapy, matrine, etoposide, resveratrol, and ergosterol have attracted the greatest research interest [39, 30, 48]. Establishing optimal dosages and compositions of potential drugs is essential for assessing the prospects for their practical use, taking into account possible interactions with other medicinal products. Sustainable cultivation and harvesting of plant sources should be ensured.

It is important to note that treatment methods based on promising plant-derived medicines do not replace conventional approaches in cancer treatment. In general, a comprehensive approach to assessing the therapeutic potential of plant-derived cancer inhibitors is required. Fungi are a vast and diverse group of organisms known for their complex chemistry and unique biological activity. Their potential to produce powerful anticancer compounds has been known for decades, leading to significant research efforts aimed at identifying and characterizing cancer inhibitors of fungal origin. On the other hand, some fungus-derived compounds can be toxic to healthy cells. Similar to plant-derived compounds, fungus-derived medicines require optimization to ensure safe and effective dosage, as well as targeted delivery. Currently, clinical trials face a number of issues associated with underfunding and regulatory obstacles. The development of safe and effective drugs based on compounds of fungal origin requires complex purification methods and the creation of appropriate formulations, as well as the introduction of innovative achievements to solve these problems. Further research is needed to elucidate the action mechanisms of these compounds and identify targeted therapeutic strategies, similar to plant-derived preparations.

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SOY LECITHIN-BASED LIPOSOMES FOR LYMPHATIC DELIVERY OF BIOLOGICALLY ACTIVE SUBSTANCES

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Introduction. The targeted delivery of lipophilic chemotherapeutic and immunomodulatory agents through the lymphatic system is a promising approach in cancer treatment. Lipid-based carriers (e.g., liposomes) are able to not only to enhance the solubility and stability of drugs, but also to ensure their protection from degradation in the gastrointestinal tract. Research into the potential of lymphatic delivery of bioactive substances using liposomes can further improve the effectiveness of lipophilic drugs.
Objective. To study the prospects for using first-generation soy lecithin-based liposomes (cholesterol-free) as a lymphatic carrier for biologically active substances.
Materials and methods. Liposomes were prepared from soy lecithin containing green fluorescent protein GFP (with a maximum fluorescence at a wavelength of 506 nm) using the method of thin-film hydration/rehydration. Some liposomes were modified by 1%, 0.5%, and 0.1% chitosan solutions. The GFP incorporation into the liposomes was visualized using confocal microscopy. *In vivo* studies were conducted in three groups of female Balb/c mice aged 11–13 weeks (three animals in each): a control group; a group receiving native fluorescent protein, and a group with the design formulation (liposomes containing fluorescent protein). After intake, the small intestine was retrieved followed by its preparation and cryosection staining. The analysis of the cell suspension was performed using a CytoFLEX V5-B5-R3 flow cytometer.
Results. The confocal microscopy study found the particle size of the liposomes obtained by the thin-film hydration method to range within 1–5 μm. The incorporation of the model protein into the liposomes, as evidenced by its content before and after the liposome formation, was at least 60%. *In vivo* experiments on mice found that intragastric administration of fluorescent protein-containing liposomes enables successful protein delivery to the intestinal wall.
Conclusions. Soy lecithin-based liposomes were obtained using the thin-film hydration method. Confocal microscopy was used to evaluate the size of the obtained liposomes and to assess qualitatively the incorporation of green fluorescent protein. The incorporation of chitosan into the liposome shell resulted in a significant aggregation of the final product, which may reduce the effectiveness of liposome delivery to cells. Confocal microscopy of cryosections and cytofluorometry of cell suspensions obtained from small intestine fragments showed the capacity of the engineered system to deliver fluorescent protein and, possibly, intact liposomes to the intestinal wall.

Keywords: liposomes; soy lecithin; delivery system; green fluorescent protein; lymphatic delivery; confocal microscopy

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ЛИПОСОМЫ ИЗ СОЕВОГО ЛЕЦИТИНА ДЛЯ ЛИМФАТИЧЕСКОЙ ДОСТАВКИ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ

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Введение. Лимфатический путь доставки может быть перспективен для липофильных химиотерапевтических и иммуномодулирующих средств, применяемых при лечении онкологических заболеваний. Носители на основе липидов (например, липосомы) могут не только повысить растворимость и стабильность лекарственных средств, но и защитить их от разложения в желудочно-кишечном тракте. Исследование возможности лимфатической доставки липосомами биологически активных веществ позволит в дальнейшем повысить эффективность многих липофильных препаратов.
Цель. Изучение перспектив применения липосом первого поколения (без холестерина) из соевого лецитина в качестве возможного лимфатического носителя для биологически активных веществ.

Материалы и методы. Получены липосомы из соевого лецитина, содержащие зеленый флуоресцентный белок GFP (с максимумом флуоресценции при длине волны 506 нм), методом гидратации/регидратации тонкой пленки. Для модификации некоторых липосом использовали раствор хитозана в концентрациях 1, 0,5 и 0,1%. Визуализация включения GFP в липосомы проведена методом конфокальной микроскопии. Исследования *in vivo* проводили в 3 группах самок мышей линии Balb/c возрастом 11–13 недель (по 3 животных в группе): контрольная группа; группа, получающая нативный флуоресцентный белок, и группа с исследуемой конструкцией (липосома, содержащая флуоресцентный белок). После введения проведен забор тонкой кишки, ее подготовка и окрашивание криосрезов. Анализ клеточной суспензии проведен на проточном цитофлуориметре CytoFLEX в конфигурации V5-B5-R3.

Результаты. При оценке полученных методом гидратации тонкой пленки липосом с использованием конфокальной микроскопии установлено, что большинство частиц имело размеры в диапазоне 1–5 мкм. Включение модельного белка в липосомы, как показали результаты измерения его содержания до и после формирования липосом, составило не менее 60%. В эксперименте на мышах *in vivo* выявлено, что внутрижелудочное введение липосом с флуоресцентным белком позволяет обеспечить доставку белка в стенку кишечника.

Выводы. Применение метода гидратации тонкой пленки позволило получить липосомы из соевого лецитина. Методом конфокальной микроскопии проведена оценка размера полученных липосом и качественно оценено включение в них зеленого флуоресцентного белка. Включение хитозана в оболочку липосом приводило к значительной агрегации конечного продукта, что может приводить к снижению эффективности доставки липосом в клетки. Конфокальная микроскопия криосрезов и цитофлуориметрический анализ клеточных суспензий, полученных из фрагментов тонкой кишки, показали, что примененная система позволяет доставить флуоресцентный белок и, вероятно, неразрушенные липосомы в стенку кишечника.

Ключевые слова: липосомы; соевый лецитин; система доставки; зеленый флуоресцентный белок; лимфатическая доставка; конфокальная микроскопия

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Исследование одобрено биоэтическим комитетом ФГУП «Научно-исследовательский институт гигиены, профпатологии и экологии человека» Федерального медико-биологического агентства (протокол № 11 от 11.02.2025).

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INTRODUCTION

When ingested, many medicinal substances (MS) can be broken down by enzymes of the gastrointestinal tract (GIT), poorly absorbed in the small intestine, and are metabolized during the first passage through the liver. For such drugs, various lipid-based micro- and nanocarriers can be used to protect them from degradation under the influence of digestive juice components, to control release, to adjust distribution, and increase bioavailability, as well as for targeted drug delivery to the site of action [1]. Drug modification by conjugation with lipids (fatty acids, glycerides, and phospholipids) increases their lipophilicity and, consequently, their bioavailability [2]. In this case, however, it is necessary to examine whether the lipid-conjugated drug equals the original drug in terms of effectiveness. In some cases, it is possible to increase the drug bioavailability by surfactants. For example, sodium caprate can increase the bioavailability of certain drugs by increasing their permeability through the intestinal epithelium via paracellular transport [3].

Over the last few years, the possibility of developing lymphatic delivery systems for various drugs has been actively studied, bypassing the hepatic first-pass effect [4–6]. This route of administration can increase bioavailability. This strategy is particularly interesting for delivering antigens to lymph nodes and enhancing the adaptive immune response induced by vaccines. Conventional systemic chemotherapy requires high doses of drugs and often proves ineffective in delivering them to the lymphatic system. The development of carriers for drugs targeting lymphocytes is a unique opportunity to increase the effectiveness of HIV/AIDS therapy [7].

At present, a large number of different carriers are known that may be promising for lymphatic drug delivery (Fig. 1). In this case, the carrier must circulate in the blood for a long time and retain the drug until its accumulation in the target organ is reached [8–10]. In addition, the MS included in the carrier should not lose its activity during circulation. When selecting a drug carrier, it should be borne in mind that the effectiveness of internalization in a cell depends on its size, shape, and charge. For example, nanosized carriers make tighter contact with biological membranes than micron-sized carriers [9]. Polymer-based drug delivery systems are not always suitable for lymphatic delivery. Some charged polymers (such as chitosan) can bind to intestinal mucosal cells through non-covalent electrostatic interactions, hydrogen bonds, and Van der Waals forces [11].

Among all lipid-based nanocarriers, liposomes show the greatest promise due to their ability not only

to encapsulate and protect drugs from degradation, but also to enhance their absorption in the intestine [4, 12]. Liposomes are spherical vesicular delivery systems containing lipids, with a phospholipid bilayer located between two hydrophilic layers [13].

The delivery mechanisms of macromolecules "packed" in liposomes may vary depending on the liposome size. Microparticles, including giant liposomes with linear dimensions similar to those of chylomicrons, are unable to penetrate blood capillaries, which have a pore size of approximately 60 nm. At the same time, the possibility of microparticles to pass through the mucous layer of the intestine has been experimentally confirmed in laboratory rodents for both biodegradable latex or polystyrene particles [14, 15] and large liposomes up to 10 μm or greater [16]. One possible way for micro-sized particles to penetrate the intestinal barrier is their absorption through microfold M cells. Although the population of M cells among the total cell number is small (~1%), they capable of transcytosis of microparticles, bacteria, viruses, lipopolysaccharides, etc. This is due to the specific features of their structure and microenvironment (reduced density of the glycocalyx, less pronounced brush border and microvilli), which determines their ability to absorb microparticles larger than 200 nm, the maximum size of lipid complexes (micelles) that can be absorbed by enterocytes. It is believed that the main function of M cells is to deliver antigens for subsequent processing and presentation to a heterogeneous population of lymphocytes, macrophages, and dendritic cells that compose the gastrointestinal immune system. However, it is known that delivering cyclosporine A in large liposomes (~10 μm) via intragastric administration in rats results in a more than nine-fold increase in this drug bioavailability [16, 17]. It should be noted that, e.g., for protein antigens, this delivery route is the most promising, allowing the antigen to be delivered directly to the lymphoid tissue of the gastrointestinal (GI) tract. When large liposomes undergo transcytosis into the submucosal layer, some of them may enter other parts of the vascular system, including the systemic circulation, through the lymphatic vessels.

In this study, we explore the potential of using first-generation (cholesterol-free) soy lecithin-based liposomes as a carrier for potential lymphatic delivery of biologically active substances.

MATERIALS AND METHODS

In this work, soy lecithin (Ultralek P, ADM, USA), recombinant green fluorescent protein zFP506 (ZsGreen1), obtained in pAAV-ZsGreen1 Vector (Takara Bio) plasmid-transfected HEK293 cells (Thermo Fisher), hexane (analytic grade, Vecton, Russia), 0.05 M solution of sodium bicarbonate (bda, Vecton, Russia), 0.05 M solution of monosubstituted sodium phosphoric acid, 0.1 M hydrochloric acid (Vecton, Russia), 0.01 M phosphate-buffer saline (PBS) — pH 7.3 containing 0.137 M NaCl and 0.0027 M KCl (Biolot, Russia), Dulbecco's solution without Ca and Mg (Biolot, Russia), bovine serum albumin (BSA) (Sigma-Aldrich), and 10% neutral formalin (Sintacon, Russia) were used.

Preparation of liposomes by thin-film hydration/rehydration

In a round-bottom flask, 100 mg of soy lecithin was dissolved in 20 mL of hexane (solvent). The resulting mixture was evaporated using a rotary evaporator (Heidolph, Germany) until a thin film was formed (water bath temperature of 45°C, pressure of 65 mbar). Green fluorescent protein (GFP) from the initial solution was diluted to a concentration of 5 mg/mL in 50 mL of 0.01 M sodium phosphate buffer (pH 7.4). The protein was incorporated into the liposomes passively during their formation from the resulting GFP solution in a flask, upon constant stirring on a magnetic stirrer (Heidolph, Germany)

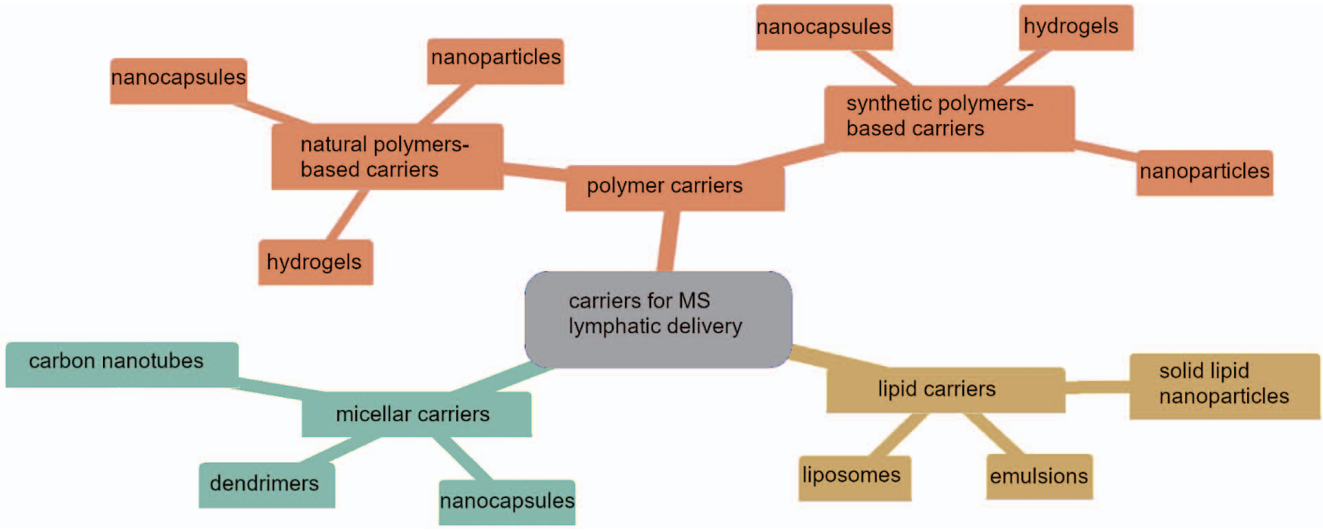


Figure prepared by the authors in the Mermaid graphical editor using data from [4–11]

Fig. 1. Promising carriers for lymphatic delivery of medicinal substances

at 400 rpm (4 h at room temperature) until the film was fully rehydrated from the walls. The obtained liposomes were centrifuged in a centrifuge (Heidolph, Germany) at a rotor speed of 20,000 rpm for 15 min to precipitate the obtained liposomes, and the supernatant was collected and washed once with deionized water.

To assess the efficiency of protein incorporation into liposomes, the protein content was determined using the Lowry protein assay in accordance with OFS.1.7.2.0023.15 in the solution before and after the liposome formation. The protein concentration in the solution decreased by approximately 60% after the process was completed, compared to the initial concentration, which should correspond to the efficiency of GFP incorporation.

Preparation of chitosan solution

A chitosan solution at concentrations of 1%, 0.5%, and 0.1% was prepared by dissolving dry samples in a 1% acetic acid solution. The pH of the resulting solutions was monitored using a pH meter; and the pH was found to be 5.4.

Preparation of liposomes by thin-film hydration/rehydration with chitosan addition

In a round-bottomed flask, 100 mg of soy lecithin was dissolved in 20 mL of hexane. The resulting lecithin solution was evaporated using a rotary evaporator (Heidolph, Germany) until a thin film was formed (water bath temperature of 45°C, pressure of 65 mbar). A 5 mg/mL concentration of green fluorescent protein was dissolved in 50 mL of 0.01 M sodium phosphate buffer (pH 7.4) and injected together with chitosan solutions of selected concentrations. The formation of liposomes was carried out under constant stirring on a magnetic stirrer (Heidolph, Germany) at 400 rpm for 4 h at room temperature until the film was completely rehydrated from the walls. The resulting liposomes were centrifuged (Heidolph, Germany) at 20,000 rpm for 15 min; the supernatant was removed; and the liposomes were washed once with water.

Confocal microscopy

Confocal microscopy was performed (Zeiss LSM 710 microscope, CarlZeiss, Germany; magnification ×63) with an argon laser $\lambda = 488$ nm. In the studied samples, GFP with the maximum fluorescence at a wavelength of 506 nm was used as a model protein for imaging liposomes.

Purification of GFP and determination of its concentration

GFP was isolated from the cell extract of HEK293 cells transfected with the pAAV ZsGreen1 Vector plasmid (Takara Bio) using the method of alcohol extraction and ammonium sulfate precipitation described in [18].

In vivo studies

The work was carried out using female Balb/c mice, which were divided into three groups of three mice in each:

- group 1 — control group;
- group 2 — animals received native fluorescent protein;
- group 3 — animals received a suspension with the studied construct (liposomes without chitosan containing GFP).

The choice of this number of experimental animals in the groups is sufficient to assess the nature and frequency of the effects recorded. The animals were 11–13 weeks old at the beginning of the experiment, and their body weight did not deviate from the average value for all experimental groups by more than 20%.

The experimental mice were injected with GFP-containing liposomes without chitosan or an aqueous solution of GFP, as described above. The control group consisted of intact mice. In cell suspensions obtained from animals, the presence of GFP fluorescence was assessed using flow cytometry.

The intragastric injection procedure was performed by qualified specialists using a probe. The injection volume was the same for all animals, equaling 300 µL. The GFP concentration in the aqueous solution used for injection into mice was determined by the Lowry protein assay, comprising 0.86 mg/mL. The same GFP solution was used in the preparation of the liposomes. In the samples studied, the efficiency of GFP incorporation into liposomes was at least 60%.

Three hours after the administration of GFP solution or GFP-containing liposomes, the mice were euthanized by cervical dislocation. After opening the abdominal cavity, a 2-cm-long fragment of the small intestine was removed 1 cm from the stomach and transferred to a separate 5-mL tube containing PBS.

A 1-cm-long portion of each intestinal fragment was separated and cut lengthwise to create a rectangular preparation.

Preparation and staining of small intestine cryosections

Uncut 1 cm long small intestine fragments were washed in PBS, cut into 0.5 cm long fragments, and embedded in Tissue-Tek® O.C.T. Compound (Sakura Finetek, Japan), filling the gel from the inside to preserve the structure of the cryosections. The samples were frozen in liquid nitrogen and sliced using a cryotome (SLEE medical, Germany). 10-µm-thick cryosections were placed on a glass slide, washed twice with PBS, and fixed in neutral formalin (3.7%) for 30 min. The fixed samples were washed twice with PBS and permeabilized with 0.1%

Triton X-100 solution in PBS with 1% BSA for 30 min, and then washed twice with PBS and 1% BSA.

To visualize cells by confocal microscopy, F-actin of the cellular cytoskeleton was stained with the Alexa Fluor 594 phalloidin fluorescent dye (Invitrogen, USA), diluted in PBS with 1% BSA in a ratio of 1:40, then 30 min and incubated in the dark. After that, the samples were washed twice with PBS and 1% BSA and enclosed in ProLong Gold antifade reagent (Invitrogen, USA). The drugs were stored in the dark at a temperature of 2–6 °C.

Flow cytometry

After washing the preparation from mucus and blood in a cuvette with PBS solution, it was transferred to a cell sieve with a 40 μm mesh size followed by addition of 1 mL of Dulbecco's solution (Biolog). The cell suspension was then obtained by rubbing. The suspension was then washed twice with Dulbecco's solution and centrifuged (8 min, 800 rpm). The washed cell pellet was resuspended in 400 μL of Dulbecco's solution.

The cell suspension was analyzed using a CytoFLEX flow cytometer in the V5-B5-R3 configuration (Beckman Coulter, USA). For each sample, 50,000 events were collected (at least 20,000 for control samples) at a sample flow rate of 10 μL/s.

The number of GFP-containing cells was counted using standard forward and side scatter detectors (to determine the morphological characteristics of the cells, such as linear size and complexity of the intracellular structure, respectively), as well as a light filter suitable for GFP visualization (FITC channel).

RESULTS AND DISCUSSION

During the study, the liposome formation technique was selected based on the following criteria:

1. Only gentle methods should be used to obtain the carrier (i.e., solvents that can alter the properties of the drug should not be used during the preparation of the delivery system; external factors such as temperature, ultrasound, high mixing rates, etc. should be minimized);
2. The particle size of the delivery system should be less than 10 μm;
3. The delivery system should contain biocompatible and non-toxic components;
4. The resulting system, after oral administration, should ensure the delivery of the packaged drug to the submucosal layer of the intestine, possibly by transport through the lymphatic vessels.

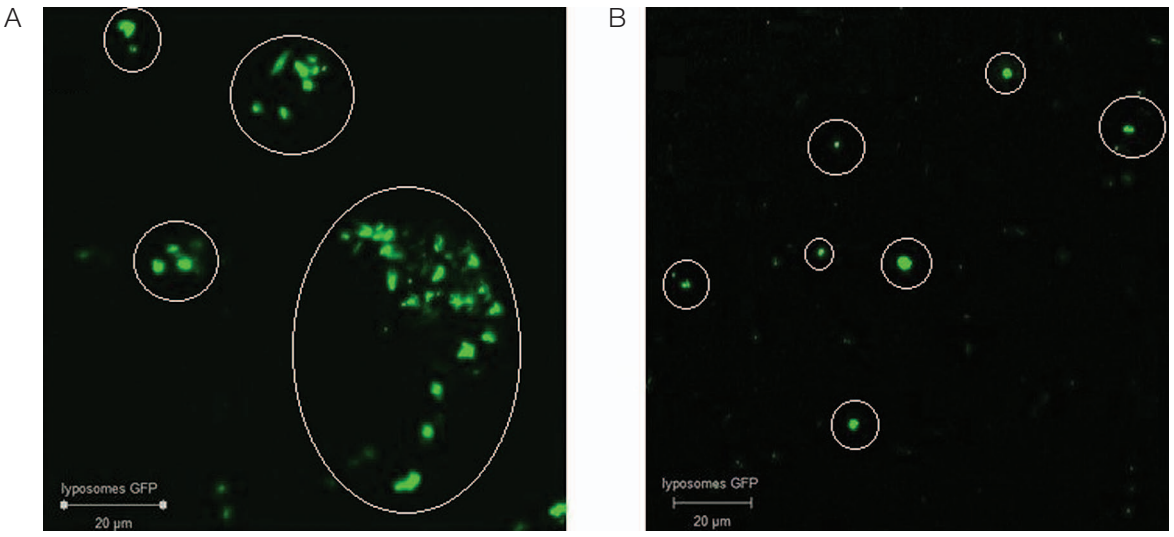
Currently, the range of various modified liposomes has been significantly extended. It is believed that polymer coatings increase the ability of liposomes to cross the epithelial barrier. Chitosans and their derivatives are widely used for this purpose. When interacting with membrane proteins, chitosan can stimulate conformational changes in protein molecules, which can lead to increased paracellular transport of biologically active molecules. However, the application of hydrophilic polymers to liposomes may not always lead to a positive effect (e.g., protection against degradation of such liposomes by digestive enzymes is not always achieved). Liposomes containing chitosan are sensitive to changes in pH and can aggregate in the gastrointestinal tract, thus deteriorating drug absorption. This effect was demonstrated in the case of oral administration of cyclosporine A incorporated into chitosan-modified liposomes [19].

During the study, two variants of liposomes were formed: those with chitosan embedded in the liposomes (to increase the mucoadhesive properties) and those without modification. Several concentrations of the polymer (1%, 0.5%, and 0.1% chitosan solution in acetic acid) were tested in the study. It was found that the introduction of chitosan into the liposomes at all selected concentrations can lead to their strong aggregation (Fig. 2A), which significantly reduces their potential as a delivery system. The most logical solution to the aggregation problem is to apply ultrasonic treatment to the particles during their formation. However, according to the selected criteria for liposome formation, this treatment was not acceptable. Therefore, we selected a delivery system without any modifications for *in vivo* studies. The liposomes obtained without chitosan had a stable linear size of 1–5 μm (Fig. 2B) and showed no aggregation.

When analyzing the cytofluorometry results, the autofluorescence level in the FITC channel, which is always present in biological objects, was taken as the value corresponding to the control group. The latter was about 1% of the parent cell population in all the samples studied (Fig. 3A). In the samples of cell suspensions from mice in the group that received GFP without liposomes, the number of events corresponding to GFP fluorescence did not exceed the level of autofluorescence in the control (Fig. 3B).

In intestinal cell samples obtained from animals in the group that received GFP in liposomes, a significant increase in events corresponding to GFP fluorescence was observed, up to 8–10% of the parent cell population (Fig. 3C).

Confocal microscopy showed that GFP molecules packed in liposomes do not break down in the stomach and can be delivered to intestinal cells (Fig. 4B). The prospects for further use of the applied delivery system depend on the effectiveness of the MS packed in liposomes and its physical and chemical characteristics. In addition, it is planned to modify the liposomes by applying Pluronic 127 to their surface. This ligand is known to improve the liposome uptake by enterocytes and increase the liposome stability. Such modification can increase the delivery system absorption by cells and ensure efficient transport through the lymphatic system. When cyclosporine A was delivered orally by liposomes with Pluronic 127, the delivery system in the gastrointestinal tract demonstrated good stability. The selected



Photos taken by the authors

Fig. 2. Confocal micrographs of GFP-liposomes: A — with chitosan coating; B — selected methodology without modifications; mark — liposomes with GFP

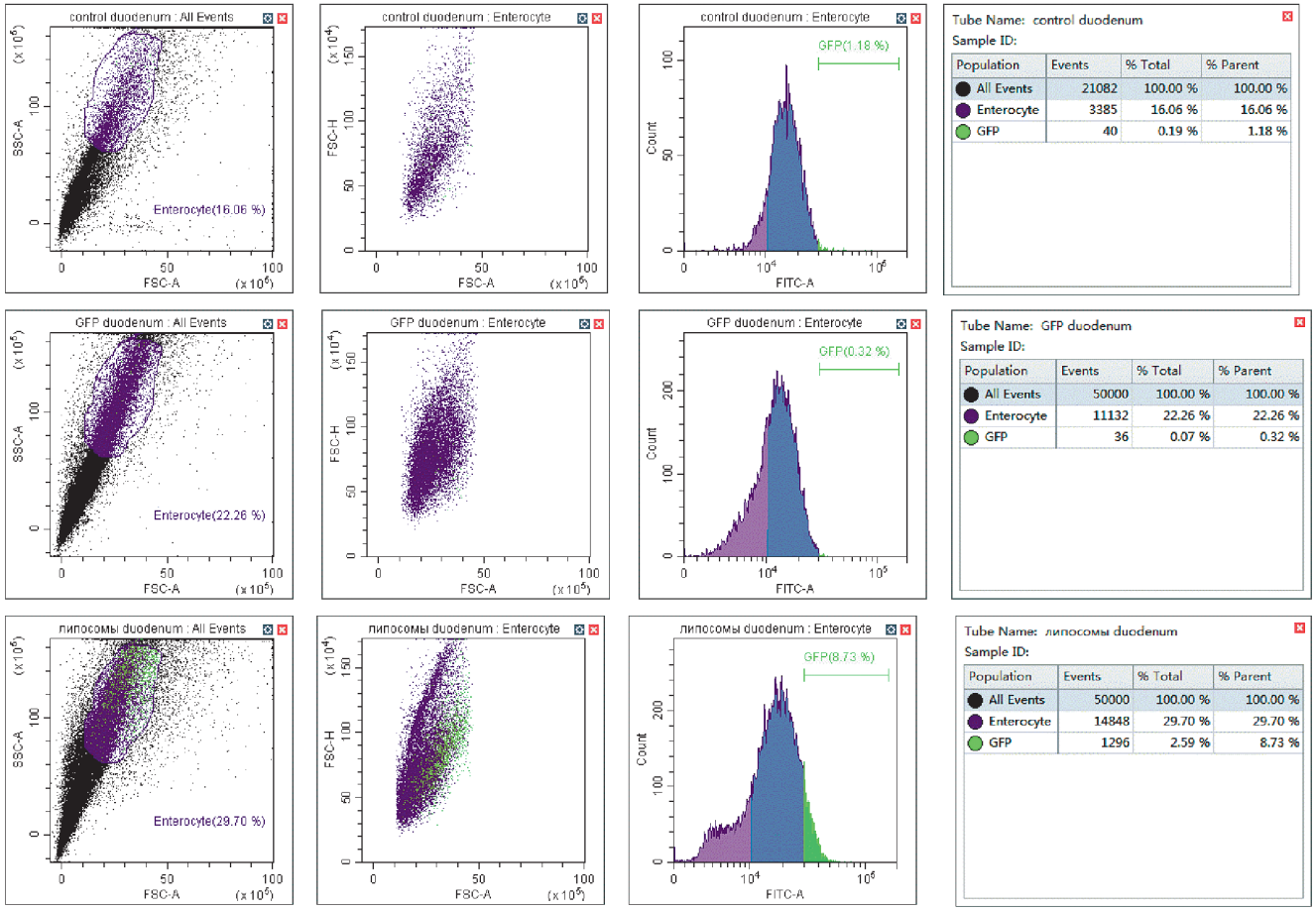
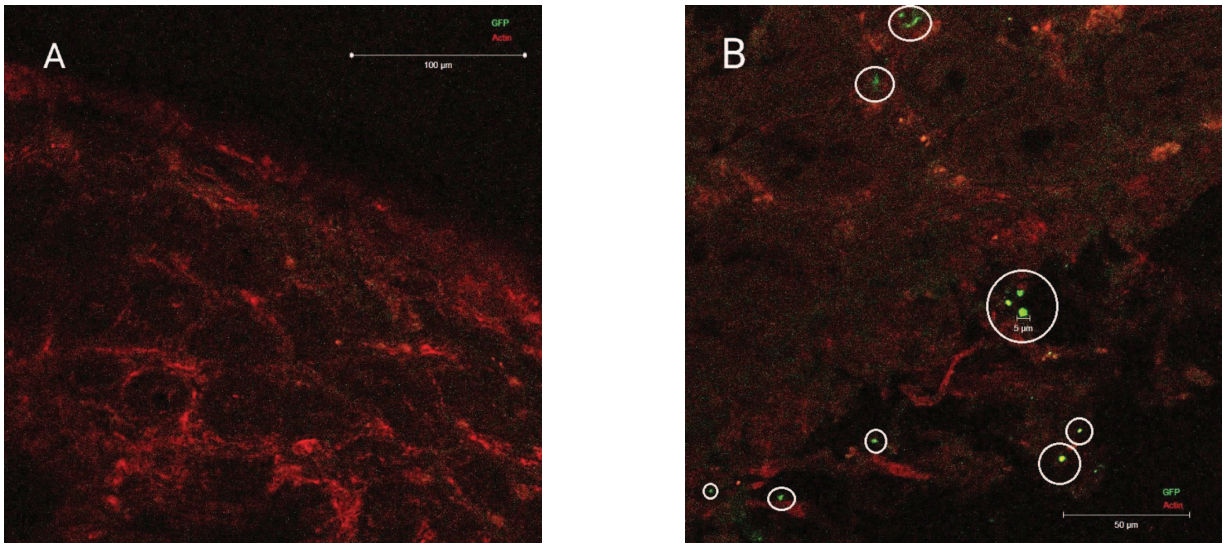


Figure prepared by the authors using their own data

Fig. 3. Flow cytometry results for GFP-liposomes

carrier reached the epithelial surface, resulting in high drug absorption [19].

The efficiency of macromolecule delivery in liposomes by oral administration is still not considered satisfactory. Improving the delivery of liposomes to cells remains an important issue in current research [20]. In that study, the authors used nanosized liposomes containing cholesterol to deliver mRNA encoding GFP to Caco-2 cells, which are often used as a model for evaluating the delivery of liposomes by



Photograph taken by the authors

Fig. 4. Results of confocal microscopy of the intestinal wall: A — after GFP injection without liposome packaging (no GFP complexes); B — intestinal wall after administration of GFP liposomes; mark — liposomes with GFP

transcytosis. There is a lack of publications on the delivery of macromolecules in liposomes using *in vivo* biologics. The results obtained in our study appear important in terms of practical assessment of the liposome use for delivering macromolecules through the gastrointestinal tract.

CONCLUSIONS

1. Soy lecithin-based liposomes were obtained by the thin-film hydration method.

2. The size of the obtained liposomes was estimated by confocal microscopy, which confirmed the incorporation of green fluorescent protein. The liposomes obtained by the selected method showed an average size of 3–5 μm.

3. It was shown that at the selected concentrations, chitosan application to liposomes can lead to significant aggregation of the final product, which in turn can negatively affect its further uptake by cells.

4. The detection of liposomes in intestinal wall cross sections indicated the possibility of their transition to the submucosal layer and the absence of their complete destruction in the gastrointestinal tract.

5. Flow cytometry confirmed the delivery of fluorescent protein to intestinal cells after intragastric administration of GFP in liposomes, which was evidenced by an increase in the proportion of cells with the GFP-corresponding fluorescence.

6. In the intestine cryosections, confocal microscopy revealed the fluorescence areas similar in size to the liposomes used in the experiment. It is likely that some of the liposomes that are absorbed through transcytosis do not undergo biodegradation for at least 3 h after administration.

7. It can be assumed that such liposomes can be used to deliver proteins or other macromolecules as antigens for immunization mediated by the cells of the gastrointestinal lymphoid tissue. However, this requires further confirmation.

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INTERNATIONAL DATA REPOSITORIES OF POPULATION-BASED IMMUNOLOGICAL AND GENETIC RESEARCH

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Introduction. Due to the active development of multiomics technologies, more and more information about human genetic and immunological research is becoming available. Data repositories are used to systematize and store such information, which facilitates the search and use of information for carrying out scientific research and solving applied problems in the area of medicine.
Objective. To analyze the global experience of using repositories of human genetic and immunological data to define their functional features and role in the development of population immunology and genetics.
Discussion. Functional features of genetic and immunological data repositories were analyzed. The data on the repositories included in the study was obtained from open sources. The selection process for repositories included three stages: selection of scientific publications, deduplication, and filtering based on selection criteria. The main criteria for the subsequent evaluation of human genetic and immunological data repositories were as follows: data volume, data accessibility, and data formats. The search for information about repositories and biobanks in the Russian Federation was conducted using online search queries on the Internet. The study analyzed 15 largest genetic and immunological data repositories, of which 37.5% are affiliated with the UK and 43.75% are affiliated with the USA. The task of creating and maintaining large repositories is solved, as a rule, by forming international and inter-institutional consortia. The availability of genetic data repositories is ensured by a combination of technological, organizational, and legal mechanisms. The most common sources of repository funding are state budgets, funds from private foundations and charitable organizations, and investments from pharmaceutical companies. The main risks associated with the operation of a repository can be divided into four groups: ethical, legal, biological, and technological risks related to data privacy. In the Russian Federation, genetic research is one of the most rapidly developing scientific directions. As a result, the challenges of secure storage, ethical use, and legal protection of data are acquiring particular importance. The presented review discusses possible directions for further development of national genetic and immunological data repositories, as well as the possibilities of additional regulation of genetic data handling at the legislative level.
Conclusions. The conducted review has identified possible risks associated with repository functioning and proposed various approaches to minimizing these risks and optimizing the development of data repositories. One of the most promising areas is the development of AI-based integration modules for processing and annotating data presented in standardized protocols.

Keywords: repository; genetic data; immunological data; scientific research; risks; biobank

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МИРОВЫЕ РЕПОЗИТОРИИ, СОДЕРЖАЩИЕ СВЕДЕНИЯ О РЕЗУЛЬТАТАХ ПОПУЛЯЦИОННЫХ ИММУНОЛОГИЧЕСКИХ И ГЕНЕТИЧЕСКИХ ИССЛЕДОВАНИЙ

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Введение. Благодаря активному развитию мультиомиксных технологий исследователи получают все больше сведений о генетических и иммунологических данных человека. Для систематизации и хранения такого рода информации используются репозитории, что ускоряет поиск и использование сведений для научных исследований и решения прикладных задач в области медицины.
Цель. Провести анализ мирового опыта эксплуатации репозиториев генетических и иммунологических данных человека для определения их функциональных особенностей и роли в развитии популяционной иммунологии и генетики.
Обсуждение. Проанализированы функциональные особенности репозиториев генетических и иммунологических данных. Данные о репозиториях, включенных в исследование, были получены из открытых источников. Процесс отбора репозиториев включал три этапа: подбор научных публикаций, дедупликация, фильтрация по критериям отбора. Основные критерии последующей оценки репозиториев генетических и иммунологических данных человека: объем данных; доступность данных; форматы данных. Поиск сведений о репозиториях и биобанках на территории Российской Федерации проводился по поисковым запросам в сети Интернет. В исследовании было проанализировано 15 крупнейших репозиториев генетических и иммунологических данных, из которых 37,5% аффилированы с Великобританией, 43,75% — с США. Для создания и поддержания крупных репозиториев, как правило, формируются международные и межинституциональные консорциумы. Доступность репозиториев генетической информации

обеспечивается комбинацией технологических, организационных и правовых механизмов. Наиболее частыми источниками финансирования репозиториев являются государственный бюджет, средства частных фондов и благотворительных организаций, инвестиции фармацевтических компаний. Основные риски, возникающие при эксплуатации репозитория, можно разделить на четыре группы: этические, правовые, биологические и технологические, связанные с обеспечением конфиденциальности данных. В Российской Федерации генетические исследования являются одним из наиболее активно развивающихся направлений науки. В этой связи становятся актуальными задачи безопасного хранения, этичного использования и правовой защиты получаемых данных. Рассмотрены возможные направления для дальнейшего развития национальных репозиториев генетических и иммунологических данных, а также возможности дополнительного регулирования обращения с генетическими данными на законодательном уровне. **Выводы.** На основании анализа данных определены возможные риски, связанные с функционированием репозиториев, предложены различные подходы к их минимизации и оптимизации развития репозиториев. В качестве одного из наиболее перспективных направлений рассматривается разработка интеграционных модулей на основе искусственного интеллекта для обработки и аннотирования данных, представленных в стандартизированных протоколах.

Ключевые слова: репозиторий; генетические данные; иммунологические данные; научные исследования; риски; биобанк

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INTRODUCTION

Currently, there is a trend towards intensive development and implementation of methodological solutions for conducting population-based immunological and genetic research in human studies. Genetic and immunological population-based studies are an interdisciplinary area that combines genetics, immunology, biostatistics, and bioinformatics. Immunology is an integral part of such studies, since genetic factors play a crucial role in shaping the immune response [1]. In addition, the study of genetic variations allows the mechanisms of autoimmune and infectious diseases to be established [2]. An important area of research is aimed at assessing the relationship between genetic heterogeneity and human health, including the predisposition to the development of various diseases [3].

The main areas of population-based immunological and genetic research include identification of genetic features of the human population (e.g., adaptation), determination of the genetic and molecular determinants of human diseases, study of the influence of genetic variability on drug response (pharmacogenetics), establishment of the mechanisms of the human immune response and assessment of its dynamical changes. Such studies employ the methods of clinical data analysis, molecular biology, immunology, and genetics. The research results are significant, first of all, for the development of applied scientific fields, such as personalized medicine aimed at developing new methods for diagnosing and assessing individual risks, creating personalized therapeutic medications, and designing disease prevention programs based on individual genetic and immunological characteristics. Currently, the leading methodological approaches in the field of population immunology and genetics are DNA and RNA sequencing, including at the level of individual cells, as well as genotyping, immunophenotyping, bioinformatics analysis, etc.

Population-based studies of genetic and immunological markers are a priority area of medical development, personalized medicine in particular. Genetic analysis is used to predict disease risks, while immune status monitoring is used to assess the effectiveness of therapeutical interventions. The data obtained can be standardized. In order to comprehensively assess a patient's health, it is necessary not only to use genetic and immunological data but also to integrate them into a unified system for analyzing variable biochemical and physiological parameters. Integration of various quantitative/qualitative medical and biological parameters into unified health assessment models require new methods and standards for variable parameters (even from the same patient), which would allow the interpretation and comparison of consolidated data. The future development of this field is largely related to technological progress: modern methods of whole-genome sequencing (including the analysis of long fragments and repeats), combined with powerful computational resources, facilitate a detailed study of the contribution of genetic variability and its interaction with various factors to the development of diseases. This not only improves our understanding of human biology, but also opens up opportunities for extending the functionality of biomedical repositories that include specialized patient databases.

The relevance of this topic is due to the high volume of human genetic and immunological data accumulated as a result of research, the need for its systematization and availability to the scientific community. According to the Science and Innovation domain, in 2010–2025 in the Russian Federation, approximately 15,000 studies mentioned genetic technologies and about 3,000 studies mentioned sequencing[1], as shown in Fig.

[1] Domain "Science and Innovation". https://gisnauka.ru/ (request date 10.04.2025).

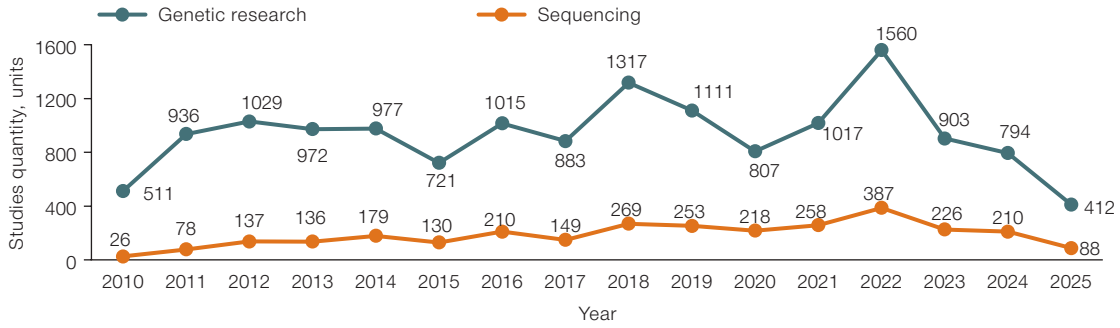

Figure prepared by the authors using the Science and Innovation domain (https://gisnauka.ru)

Fig. Dynamics of research and development activities in 2010–2025

At present, repositories for storing genetic and immunological data are being formed all around the world. The main objective of these repositories is to accumulate and systematize data related to genetic variability, in order to subsequently analyze and develop new methods for diagnosing and assessing individual risks, creating personalized medications for therapy, and developing disease prevention programs based on individual genetic and immunological characteristics.

The creation of large genetic repositories is associated with a set of interrelated problems. First, there are acute ethical issues, such as obtaining informed consent from the patient, ensuring strict confidentiality of their data, and informing the patient about precautions to protect genetic information on their personal devices. Second, there are analytical and technical challenges related to the ever-increasing volume of data, which requires continuous improvement of methods for its interpretation and annotation, as well as the development of approaches for comprehensive analysis within various scientific concepts. This, in turn, necessitates the resolution of data management issues, including the establishment of clear access rules for qualified personnel and the provision of reliable information security. Finally, it is important to consider the biological complexity when interpreting the results; thus, the implementation of genetic information into phenotypic traits is always modulated by multiple environmental factors.

In this study, we aim to review the global experience of using human genetic and immunological data repositories with the purpose of determining their functional features and role in the development of population immunology and genetics. To that end, the following main objectives were formulated: to analyze the global experience of practical application of information stored in human genetic and immunological data repositories in order to determine the functional features of such repositories; to identify possible risks, including ethical risks, risks of violating personal data confidentiality, and unauthorized use risks, as well as the data interpretation correctness and reliability; and to identify possible ways to mitigate these risks, development of proposals to improve the implementation of genetic and immunological research results in practical medicine.

MATERIALS AND METHODS

We carried out an analysis of existing genetic and immunological data repositories based on information obtained from open sources. To that end, 15 repositories were selected based on the highest levels of peer review in the professional community, the frequency of their mentioning in scientific publications, data from consortia, and an analysis of official web resources. The selection process for repositories included a three-step strategy: the selection of scientific publications, deduplication, and filtering based on selection criteria.

The search process conducted through PubMed and Google Scholar produced more than 100 studies published in 2018–2024. The keyword phrases were “genetic data repository” and “immunological data repository”.

The initial pool of publications was analyzed and sorted by the frequency of references, resulting in the formation of a preliminary list of repositories and the removal of duplicate repositories.

Next, repositories with insufficient description or closed/paid access were excluded. In the next stage of selection, the following criteria were taken into account: data volume (more than 500 values), geographical representation (the study included repositories from four countries: the USA, the UK, Spain, and the Netherlands), degree of data availability (open/closed/paid access or only for the founder’s employees), application of international standards for data storage (repositories containing data in the most common formats, such as vcf).

For further analysis, the main criteria for evaluating human genetic and immunological data repositories were established, namely, data volume (the amount and diversity of stored data); data accessibility (direct access, licensing); and data formats (supported file formats).

To analyze the activities of the repositories, we selected the resources that were formed in various countries and were active at the time of the study.

We searched for information about repositories and biobanks in the Russian Federation on the Internet using the following search terms: “genetic data repository” and “immunological data repository”.

Despite the existence of numerous repositories, the resources included in the study were the most significant and sought-after systems for the scientific community. The limitation of the analyzed sample is due to the need to include only repositories with well-documented data. The final sample covered global and niche platforms that are relevant for further analysis of genetic or immunological information in scientific research.

RESULTS AND DISCUSSION

The list of selected repositories included 15 information resources: IGSR, AFND, GWAS, gnomAD, NCBI GEO, BBMRI-NL, FHLdb, AIRR Data Commons, EGA, IEDB, OMIM, NIDDK Central Repository, ArrayExpress, ENA, and dbGaP [4–16]. Among them, 1 (6.25%) repository was created in the Netherlands, 2 (12.5%) in Spain, 6 (37.5%) in the UK, and 7 (43.75%) in the USA.

As a rule, large repositories are created and maintained by forming international and inter-institutional consortia. The most common sources of funding for repositories are government budgets (which account for a significant portion), private foundations and charities, and investments from pharmaceutical companies. For example, in the USA, research funding is provided by the National Institutes of Health (NIH), with contributions from private foundations and charities. Pharmaceutical companies also provide funding, due to their interest in the results of genetic research for the development of new medications.

Genetic and immunological population-based studies are the fundamental basis for the development of modern medicine, including new methods for the disease diagnosis, therapy, prevention.

The availability of repository data is a key condition for conducting scientific research; thus, such information ensures reproducibility, enables comparative analysis, and supports the development of new tools and methods. It is the direct access to data that encourages the creation of new algorithms and software for data analysis. The availability of genetic data repositories is ensured by a combination of technological (standardized data formats, technical infrastructure), organizational (metadata and annotations), and legal mechanisms (personal data and ethical aspects). Currently, the most common form of data access is through web interfaces. This access can be free or paid (licensed). For example, data from the IGSR is publicly available and can be freely distributed [4].

Profile of leading genetic repositories

A review of open sources was conducted to compare human genetic and immunological data repositories. According to the pre-defined evaluation criteria, 15 leading repositories with significant data sets were identified. A list of aggregated data was compiled for each selected repository, which is presented in the Table. The list also includes references to the underlying sources that describe the structure, purpose, and functioning of the repositories, provided that such references are available on their official websites.

Among the 15 repositories listed, 1 (6.25%) was developed in the Netherlands, 2 (12.5%) in Spain, 6 (37.5%) in the UK (1 of which was developed jointly with Spain), and 7 (43.75%) in the USA. In the Table, 12 (80%) are direct-access repositories. Although 3 (20%) repositories provide closed access, detailed information on their activity is available. In the majority of cases, the data for export is presented in the txt format. However, it should be noted that the list includes only the largest and most widely used repositories, not all those available globally. These repositories stand out not only in terms of their data volume, but also in terms of their openness, which facilitates international collaboration and improves the reproducibility of research.

It should be noted that some repositories provide access to only portions of the data. However, closed information resources that specialize in storing confidential data are also useful for research purposes. These databases, upon agreement with their owner, provide access to clinical, phenotypic, and genetic data, making them essential for medical research that requires privacy protection. Additionally, there are closed repositories that are not included in the list.

General principles for organizing genetic repositories

Our study found the following essential conditions for a high-quality repository:

- standardization, i.e., the data stored in repositories must comply with specific standards to ensure proper processing, comparison, automated analysis, and long-term storage;
- reproducibility, including the importance of uniform file formats;
- the presence of experimental conditions and metadata describing the data collection methodology, the samples used, and the analyses performed.

Data storage formats

We identified a high-level heterogeneity among experimental platforms and analysis methods, which leads to a variety of data formats. This is primarily due to the human factor, since different specialists use different methodological approaches to solve the same bioinformatics problem. In the future, this could lead to the impossibility of data comparison.

At the same time, standardized formats and ontologies are used to ensure data compatibility when

Table. Aggregated data on repositories

No.	Repository name	Prospect	Description	State	Data set	Data accessibility	Data export formats
1	The International Genome Sample Resource (IGSR)[2]	Genetics	A catalog of common human genetic variations, including samples taken with the consent of individuals	UK	2504 samples from 26 populations	Direct access	VCF, Fastq, BAM
2	Allele Frequency Net Database (AFND)[3]	Genetics, immunology	Database containing allele frequencies of immune genes and their corresponding alleles in various populations	UK	The number of frequencies: 155,685 (HLA), 6731 (KIR), 4376 (cytokine), 877 (MIC) from 14,264,290 people. Population studies — 1802 people, gene/allele data — 1786 people, haplotype data — 684 people, genotype data — 192 people	Direct access	CSV
3	The NHGRI-EBI Catalog of human genome-wide association studies (GWAS)[4]	Genetics	Contains information about associations of genetic markers with phenotypes useful for pharmacogenetics	UK	7083 publications, 692,444 primary associations, and 96,947 complete summary statistics. Data is mapped to Genome Assembly GRCh38.p14 and dbSNP Build 15	Direct access	TSV, OWL/RDF, EFO
4	The Genome Aggregation Database (gnomAD)[5]	Genetics	Provides exome sequences and complete genomes for studying rare genetic variants	USA	730,947 exome sequences and 76,215 complete genome sequences from unrelated individuals of different origins	Direct access	VCF, TSV
5	Gene Expression Omnibus (NCBI GEO)[6]	Genetics	Database for gene expression profiling and RNA methylation profiling	USA	4348 data sets	Direct access	TXT, XML, SOFT, MIAME.
6	Biobanking Netherlands (BBMRI-NL)[7]	Genetics	A set of data and “omic” signatures of diseases	Netherlands	Genetic, epigenetic, transcriptomic, and metabolomic data from 35,000 samples from 29 cohorts	Closed access	TXT

[2] IGSR: The International Genome Sample Resource (project 1000 Genomes). http://www.internationalgenome.org/ (request date 27.11.2024).
[3] Allele Frequency Net Database. http://www.allelefrequencies.net (request date 27.11.2024).
[4] GWAS: The NHGRI-EBI Catalog of human genome-wide association studies. https://www.ebi.ac.uk/gwas/ (request date 27.11.2024).
[5] gnomAD: The Genome Aggregation Database. https://gnomad.broadinstitute.org/ (request date 27.11.2024).
[6] NCBI GEO: Gene Expression Omnibus. https://www.ncbi.nlm.nih.gov/geo/ (request date 27.11.2024).
[7] BBMRI: Biobanking Netherlands. https://www.bbmri.nl/ (request date 28.11.2024).

Table (continued)

No.	Repository name	Prospect	Description	State	Data set	Data accessibility	Data export formats
7	Database on the Molecular Basis of Familial Hemophagocytic Lymphohistiocytosis (FHLdb)[8]	Genetics, immunology	Database of variants of familial hemophagocytic lymphohistiocytosis	Spain	Information about registered variants in 4 FHL-related genes (*PRF1*, *UNC13D*, *STXBP2*, *STX11*), including 579 variants (including missense, nonsense, indel, splicing, etc.)	Direct access	JSON
8	AIRR Data Commons[9]	Genetics, immunology	Data on the use of sequencing technologies to study the repertoires of antibodies/B-cell receptors and T-cell receptors	USA	5.2 billion annotated sequences, 67,000 clones, 133,000 sorted, single B/T cells	Direct access	MIAIRR, YAML/ JSON
9	European Genome-phenome Archive (EGA)[10]	Genetics, immunology	Archiving and dissemination of personal identifiable genetic and phenotypic data	UK, Spain	11,775 genetic, phenotypic, and clinical data sets	Closed access	TAR.GZ
10	The Immune Epitope Database (IEDB)[11]	Genetics, immunology	A resource for searching and exporting immune epitopes	USA	Peptide epitopes: 1,621,303; non-peptide epitopes: 3189; T-cell analysis: 541,542; B-cell analysis: 1,414,095; MHC ligand analysis: 4,881,627; epitope source organisms: 4579; references: 25,400	Direct access	XLSX, CSV, TCB, JSON
11	Online Mendelian Inheritance in Man (OMIM)[12]	Genetics	An updated catalog of genes, genetic disorders, and phenotypic traits in humans and their relationships	USA	Autosomal genes: 26,080; X-linked genes: 1382; Y-linked genes: 63; mitochondrial genes: 72	Direct access	TXT
12	Central Repository — National Institutes of Health (NIDDK)[13]	Genetics	A centralized research resource for diabetes, digestive system diseases, and kidney diseases	USA	5767 data sets	Closed access	CSV

[8] FHLdb: Database of variants of Familial Hemophagocytic Lymphohistiocytosis syndrome. https://www.biotoclin.org/FHLdb/ (request date 28.11.2024).
[9] AIRR Data Commons. https://docs.airr-community.org/en/stable/index.html (request date 28.11.2024).
[10] EGA: European Genome-phenome Archive. https://www.ebi.ac.uk/ega (request date 28.11.2024).
[11] IEDB: The Immune Epitope Database. https://www.iedb.org/ (request date 28.11.2024).
[12] OMIM: Online Mendelian Inheritance in Man. http://www.omim.org/ (request date 28.11.2024).
[13] NIDDK Central Repository — National Institutes of Health. https://repository.niddk.nih.gov/home/ (request date 29.11.2024).

Table (continued)

No.	Repository name	Prospect	Description	State	Data set	Data accessibility	Data export formats
13	Functional genomics data (ArrayExpress)[14]	Genetics	Collection of functional Genomics data	UK	78,511 data sets	Direct access	CEL, TXT, XML
14	European Nucleotide Archive (ENA)[15]	Genetics	A resource of bio-data, including nucleotides. A repository providing access to annotated DNA and RNA sequences, to information on experimental procedures, etc.	UK	Assembly 2,046,549; Sequence 23,430,609; Coding 38,244,291; Non-coding 1,265,026; Read 2,990,747; Analysis 998,067	Direct access	CSV
15	Database of Genotypes and Phenotypes (dbGaP)[16]	Genetics	Database of genotypes and phenotypes	USA	Genotype 4,039,007; Expression Analysis 422,847; Somatic Mutations 100,614; Genome 683,996; Epigenome 88,733	Direct access	TXT

Table prepared by the authors

exchanging information between different repositories. For example, the FASTQ format is used to store sequencing read sequences; SAM is a text format for storing aligned sequences; bam is a binary format for storing aligned sequences; VCF is a more compact format than SAM for storing and analyzing large amounts of sequencing data; TXT (text format, tab-delimited) is the simplest and most common format, containing expression values for each gene in each sample, and is suitable for importing into most data analysis programs; CSV (comma-separated values) is similar to TXT, but separates values with commas, and is widely supported; SOFT (simple omnibus format in text) is a more structured format than TXT/CSV, which contains metadata about the platform, samples, and gene expression data, allowing for automated processing and analysis of large amounts of data, it is generally recommended for comprehensive analysis; MIAME (minimum information about a microarray experiment) is an XML-based format that contains more metadata than SOFT, ensuring maximum reproducibility and interpretability of data, it is often used for data exchange between databases and for analysis purposes; TCB and EFO are files for use in Binary Data or other software; RDF is a format for representing interrelated data; XML is a markup language that allows the user to define and store data; MiAIRR is a data set that defines the information that should accompany TCR/BCR repertoire data for its correct interpretation; YAML is a structured representation of information; JSON is a standard text format for storing and transmitting structured data; TAR.GZ is an archive of data; XLSX is a table-based data format; CEL contains all types of data that have been collected; TSV is a text format for representing database tables; OWL is an ontology description language.

Repository components and structure

A repository typically consists of several components: a database, which is the central repository of information, where data such as DNA sequences, gene expression profiles, phenotypic data, and metadata and ontologies are stored; a web interface that allows the user to search, filter, download, visualize, and analyze data; a version control system that allows the user to track changes in data and metadata and restore previous versions when necessary; an access control system that determines which users have access to which data, which is especially important for protecting the privacy of personal data.

Metadata can be of various types. Thus, some repositories contain data only on the country of

[14] ArrayExpress: Functional genomics data. https://www.ebi.ac.uk/biostudies/arrayexpress (request date 27.11.2024).
[15] ENA: European Nucleotide Archive. https://www.ebi.ac.uk/ena/browser/home (request date 29.11.2024).
[16] dbGaP (Database of Genotypes and Phenotypes). https://www.ncbi.nlm.nih.gov/gap/ (request date 29.11.2024).

residence of the people participating in the study, while others may contain more detailed data, such as the age, gender, and ethnicity of the people participating in the study. Additionally, there are resources dedicated to visualizing genetic data, known as genomic browsers.

The structure of databases can be centralized (accessible to a wide range of researchers; this approach is typically used in large international projects) or decentralized (accessible only to a limited number of users; this approach is used in many research institutions and universities). Additionally, it should be noted that consortia can be formed to unite multiple research groups to create and maintain large databases. An important element of any repository structure is the preservation of data over a long period of time, which is especially important for long-term research. Currently, cloud technologies are being used to store genetic and immunological data.

Genetic databases as the foundation of modern genomic research are extensive collections of information about the human genome and other organisms, including variations in DNA sequences, their frequency in different populations, and their association with phenotypic traits. These databases can be divided into two main subcategories: general genomic databases (e.g., the 1000 Genomes Project) and genotyping and phenotyping databases (e.g., NHGRI GWAS) [6].

Immunological databases (e.g., IEDB) play a key role in understanding the mechanisms of the immune system, which is a complex network of cells, tissues, and molecules that protect the body from infections and other threats. These databases can be divided into the following categories: immune receptor databases and immunophenotyping databases [13].

Multimodal databases are integrative databases obtained by various omics technologies, including genomics, transcriptomics, proteomics, and metabolomics. The application of such databases provides a more complete understanding of biological processes and complex interactions between different levels of biological organization (e.g., as applied in GEO) [8].

Repositories usually provide direct access to data, although there may be restrictions related to privacy or copyright. There are also mixed-type repositories that support direct access to some, while closed or paid access to other data.

In some repositories, users can search for data based on various criteria (such as data type, organism, or disease) and download it for further analysis.

Data privacy

Data privacy is a critically important issue for genetic and immunological repositories. To ensure data privacy, a range of measures are taken to protect the personal information of research participants. The key measures for ensuring data privacy include data anonymization; data aggregation, where data from multiple individuals is combined to create larger groups, making it difficult to identify individual participants; data encryption during storage on servers and transmission between systems; and a multi-level access role model; multi-factor authentication; actions logging; detection of unauthorized access signs; privacy agreements; regular security checks for vulnerabilities; additional measures applied to limit the duration of data storage.

Repository operation risks

The main risks associated with the use of repositories are of ethical, legal, and biological origins [17]. It is important to note the ethical risks are associated with obtaining genetic and immunological data in research. For example, when conducting research on the genomes of indigenous peoples in coastal Ecuador [18] and American Indians [19], given the highly specific and unique characteristics of the study cohorts, there is a risk of data leakage even with the use of the most advanced anonymization methods. Genetic data misuse can also lead to false conclusions and discrimination. The discovery of genetic markers associated with certain diseases can contribute to social stigmatization. Additionally, the monopolization of genetic and immunological data can limit access to important medical research and development.

Among other things, the issue of protecting intellectual property rights for data acquisition methods and genetic and immunological data itself is at risk. Genetic data is often considered to be a discovery of natural phenomena rather than an invention, which makes it difficult to patent. However, genetic data is regularly updated and expanded, which creates challenges when defining the boundaries of intellectual property. It is also worth noting that there are currently significant differences in the legislation of different countries, which makes it difficult to protect the intellectual property of genetic and immunological data internationally. The intellectual property protection based on genetic and immunological data may conflict with other rights, such as the right to privacy and the right to information. There are also technological challenges, such as the identification and data tracking and the protection against unauthorized use. Possible solutions to these problems include:

- establishing independent ethical committees to evaluate projects involving genetic data;
- raising public awareness about the importance of protecting genetic and immunological data and the related ethical issues;
- regulating direct access to genetic data to accelerate scientific research and prevent monopolization;
- developing adaptable licensing agreements that ensure a balance between protecting intellectual property and ensuring public access;

- establishing international accords and standards to regulate the protection of intellectual property rights to genetic data.

The main biological risks are associated with the uncontrolled release of genetically modified food products; editing of the human genome; and the creation of biological weapons [17]. In Russia, the procedures for the release of genetically modified food products are based on licensing, certification, and registration of genetically modified organisms, as well as their control. The Criminal Code of the Russian Federation defines the provisions governing the responsibility for the creation and use of biological weapons.

Genetic repositories in the Russian Federation

Russian genetic and immunological data repositories were not included in the study due to their non-compliance with the selection criteria. However, these repositories should be mentioned to provide a comprehensive understanding of the development of this area in the Russian Federation.

The Russian Federation is actively supporting genetic research, which raises a number of important issues related to the storage, use, and protection of genetic data. In 2019, the Federal Research Programme for Genetic Technologies Development[17] was approved. This Programme aims to promote the development of genetic technologies in Russia. As part of the Programme, three world-class genomic research centers have been established.

There are several open genetic repositories available for researchers in Russia. In 2021, the Genetico center and the Serbalab laboratory, in collaboration with the Bioinformatics Institute, created and made publicly available the first Russian database of genetic variants and their occurrence in the Russian population, referred to as RUSeq. This database contains information about genetic variants identified in more than 6,000 individuals. It is important to note that, similar to most foreign repositories, RUSeq stores de-identified data [20].

There is also the National Aggregator of Open Repositories of Russian Universities (NORA)[18], which combines the research results of Russian researchers and provides access to materials published in the public domain. The Vavilov All-Russian Institute of Plant Genetic Resources (VIR) also collects, stores, and studies plant genetic resources and provides access to the VIR collections for scientific research.

In 2024, the Law on the creation of the National Genetic Information Database was enforced[19]. This is a state information system for ensuring national security, protection of life and health of citizens. It guarantees sovereignty in the field of storage and use of genetic data, as well as the exchange of information between governmental agencies and holders of relevant information. This will make it possible to conduct large-scale genetic research and develop new methods for the diagnosis and treatment of diseases, to develop the pharmaceutical industry, and to improve the quality of medical care.

In 2020–2024, the Russian Federal Medical and Biological Agency (FMBA) developed one of the world's largest databases of population frequencies of genetic variants.[20] This database contains data on 120,000 conditionally healthy people, as well as information on more than 550,000,000 unique genetic variants and their prevalence in the Russian population. It should be noted that the structure of this database is centralized and similar to international repositories.

The National Genetic Initiative "100,000+Me" is a unique Russian project aimed at improving the methods of diagnosis and therapy of hereditary and oncological diseases by determining the genotypes of 100,000 Russians from various geographical regions and different populations. The aim is to search for genetic variants that occur in Russia, summarize their similarities, and identify differences.[21] The "100,000+Me" initiative is being implemented by "Biotek Campus" and was developed jointly by Rosneft Oil Company and Lomonosov Moscow State University.

A special mention should be made of the Russian resource implemented by the Federal Medical and Biological Agency (FMBA) of Russia — the National Information Resource, which contains information about population-based immunological and genetic studies conducted in the Russian Federation. Information about such studies contains the results of research aimed at obtaining information that is inextricably linked to the molecular and genetic characteristics of humans, which contribute to the study of health parameters, prediction of the risk of developing chronic diseases, and assessment of the functional characteristics of the human immune system in normal and pathological conditions. Around the world, there are a number of disconnected repositories containing genetic and immunological data; however, the FMBA resource is a unique solution that provides a comprehensive analysis of a large number of studies in a single digital interface. In the future, this resource may facilitate international collaboration between research teams based on the analysis of their competencies and experience in the application of advanced methods for conducting genetic and immunological population-bases studies. This is important for advancing scientific research in the area of healthcare at

[17] Federal Research Programme for Genetic Technologies Development for 2019–2027. http://government.ru/docs/36457/ (request date 29.11.2024).
[18] National aggregator of open repositories of Russian universities (NORA). https://www.openrepository.ru/ (request date 29.11.2024).
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[20] Database of population frequencies of genetic variants of the population of the Russian Federation. https://nir.cspfmba.ru/info (request date 29.11.2024).
[21] National Genetic Initiative "100,000+Me". https://www.biotechcampus.ru/ (request date 29.11.2024).

the national level. Currently, there are other analogues in the Russian Federation.

In addition, in Russia, a network of biobanks is currently being developed. A biobank is a repository that contains human biological samples (blood, saliva, and tissues) and related genetic data. Each biobank requires quality control. For example, such biobanks have been established at the following facilities: National Medical Research Center for Therapy and Preventive Medicine[22], Almazov National Medical Research Center[23], and the Sechenov First Moscow State Medical Univesity[24]. Biosamples from these biobanks are used for various research projects, including the study of genetic biomarkers for diseases and the development of new medications.

In this study, we analyzed information exclusively from open databases, without taking into account repositories with closed or paid access for Russian organizations, which comprises the main limitation. It should also be noted that our objectives did not include analyzing and identifying the features of all existing repositories, which determined the inclusion of only a few repositories with a large amount of well-documented data.

The standardization of genetic data repositories plays a key role in ensuring compatibility, reproducibility, and efficient use of information. Thus, unified storage formats such as FASTQ for sequences, VCF for genomic variants, and BAM/SAM for alignments minimize the risk of errors during analysis, while support for cloud integrations and open APIs (Application Programming Interface) allows for research scaling without the need for manual downloading of large amounts of data. Metadata standards ensure that samples are described in full (source, sequencing methods), which is important for comparing results between different studies. Unified bioinformatics protocols reduce variability in data processing. Without this standardization, it would be impossible to integrate large-scale projects such as IGSR with clinical databases, which would hamper the identification of pathogenic mutations and the development of personalized medicine. Currently, a number of studies are being conducted to optimize the storage formats for genetic data [21] and the protocols for their bioinformatics processing [22].

It should be noted that the joint storage of genetic and immunological data is successfully implemented in the following repositories: Allele Frequency Net Database, FHLdb, AIRR Data Commons, EGA, and IEDB. It is important to emphasize that the development of a unified global repository with the ability to integrate modules containing standardized results of genetic and population immunological studies can have a significant positive impact on the development of medicine and accelerate technological progress.

Ethical regulation of genetic research aims to provide a balance between scientific progress and the task of human rights protection, including the mandatory obtaining of informed consent, guaranteeing the data anonymity, and preventing possible discrimination. Special attention is paid to vulnerable groups (indigenous peoples, patients with rare diseases), whose data can only be used if they are directly involved in decision making. Modern challenges, such as the risk of re-identification of anonymous genomes or the use of artificial intelligence in DNA analysis, require constant updating of legal norms and strengthening of cybersecurity to maintain public trust in genetic research. There is a view that ethical committees should acquire a more significant weight than they currently do [23]. It is important to note that protection against ethical risks is a relevant topic of discussions both in Russia and abroad.

In the Russian Federation, genetic information and its legal regime are regulated by the Federal Law[25], which defines genomic information as personal data that includes encoded information about certain fragments of a person's or an unidentified corpse's deoxyribonucleic acid, which does not characterize their physiological characteristics. Additionally, Federal Law No. 152-FZ of July 27, 2006[26], establishes that genomic information is closely related to the category of biometric personal data (article 11) and requires the individual's consent for its subsequent processing and dissemination (article 7). However, there is an opinion that it is incorrect to refer to genetic information only as personal data, since the owners of this information are not only the persons who participated in the study, but also their genetic relatives [24]. In this regard, it seems appropriate to define genetic information as an independent type of data at the legislative level. Once such a definition is established, there will be a need for further legislative regulation of the handling of genetic information. It is worth noting that many Russian authors of scientific publications emphasize the need to improve the regulatory framework, e.g., in terms of genetic passporting of the population [25], protection of genetic data [26], or the development of genomic law [27].

Patenting genetic sequences as substances is acceptable; however, it carries the risk of duplicating rights to identical sequences, which requires limiting absolute protection. A possible alternative is to patent a specific application of the sequence (such as a therapeutic method), which requires international harmonization due to conflicts of interest. Current regulations focus

[22] Biobank at the National Medical Research Center for Therapy and Preventive Medicine. https://gnicpm.ru/scientific-directions/biobank.html (request date 29.11.2024).
[23] Biobank at the Almazov National Medical Research Center. http://www.almazovcentre.ru/?page_id=69701 (request date 29.11.2024).
[24] Biobank at the Sechenov First Moscow State Medical Univesity. https://www.sechenov.ru/pressroom/news/zamorozhennaya-kollektsiya-kak-sechenovskiy-universitet-sozdaet-biobank-dlya-nauchnykh-issledovaniy-/ (request date 29.11.2024).
[25] Federal Law No. 42-FZ dated December 3, 2008 "On State Genome Registration in the Russian Federation" (as amended and supplemented).
[26] Federal Law No. 152-FZ dated July 27, 2006, "On Personal Data".

on industrial applicability, requiring the disclosure of the functional annotation of a gene, rather than solely its structure. Although the know-how regime is possible, it restricts access to data, hindering scientific innovation, and is less attractive due to the lack of exclusive rights. There is also a view that a clearer distinction between "discovery" and "invention" in the genetic research area is necessary [28]. Most legal systems, including the Russian one, currently do recognize the fundamental possibility of patenting a gene (or a gene fragment), but under certain conditions that aim to prevent the monopolization of knowledge about nature.

CONCLUSIONS

In the long term, the development of genetic and population-based immunological research will significantly improve public health and enhance the quality of life. International genetic data repositories play a crucial role in advancing modern medicine and scientific research by providing access to extensive databases for studying genetic variation, disease susceptibility, and treatment responses. However, in order to effectively use this data, it is necessary to address the challenges of improving the quality and security of stored information, ensuring confidentiality, and standardizing methodological solutions to facilitate analysis and interpretation of data by a wide range of specialists, including those without programming skills (molecular biologists, medical doctors, etc.). The continuous development of technology and the emergence of new standards are making repositories an increasingly effective and user-friendly tool. From the point of view of repository evolution, the development of an integration module for use in processing and annotating artificial intelligence (AI) data with standardized protocols seems promising. The use of such a module for data annotation and analysis automation will accelerate the information processing and increase the efficiency of using arrays of stored data. In addition, the development of technologies for confidential data analysis, such as federal AI training, will provide the possibility of data sharing without its transfer to third parties. Improving data repositories facilitates the implementation of genetic and immunological research findings into practical medicine.

The significant amount of genetic data accumulated in Russia as a result of approximately 15,000 research projects conducted in 2010–2025 highlights the urgent need for the creation and development of national genetic and immunological repositories. Effective use of this data is crucial, since the analysis of genetic and immunological information provides a deeper understanding of disease mechanisms, enhances risk assessment, and enables the development of new diagnostic, preventive, and therapeutic approaches, as well as the creation of innovative medications. In order to increase the international competitiveness and contribution of Russian repositories to global research, it is necessary to provide mechanisms for partial direct access to anonymized and aggregated data from domestic repositories, as well as to explore the possibilities of integration with international databases.

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ACE2 GENE TRANSGENESIS ENHANCES MEMORY OF PSYCHOPHYSIOLOGICAL TRAUMA IN MOUSE MODELS OF POST-TRAUMATIC STRESS DISORDER

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Introduction. The development of symptoms in post-traumatic stress disorder (PTSD) is determined by a set of factors, which are not limited to classical neurotransmitter systems in the brain or stress hormones. In particular, the brain renin-angiotensin-aldosterone system may be involved in the mechanisms of PTSD.
Objective. To study the effect of *hACE2* expression, angiotensin-converting enzyme 2 (ACE2) gene, on anxiety and susceptibility to psychophysiological stress in mice in the foot electroshock (FS) model of PTSD.
Materials and methods. The experiments were conducted using 4–5-month-old male C57Bl/6N and k18-hACE2-KI mice. C57Bl/6N mice were divided into three groups: control (n = 7); the foot shock (FS) (n = 7); FS + lisinopril (n = 7). k18-hACE2-KI mice were divided into two groups: control (n = 7) and the FS (n = 8). Pavlovian fear conditioning was performed using FS as an unconditioned stimulus. Mice in the FS + lisinopril group received lisinopril at a dose of 10 mg/kg per day with drinking water for 28 days after psychophysiological trauma. The expression of fear, reflecting the memory of psychophysiological trauma, was assessed on day 7 and day 28 after FS exposure. The magnitude of the fear response was assessed by evaluation of the relative time of freezing. The open field test was used to assess general locomotor activity. The tail suspension test was used to assess the stress-coping strategy, while the light-dark box test and the elevated plus maze test were used to measure anxiety. The Barnes maze test was used to explore spatial navigation and spatial learning dynamics. Behavior was analyzed using the ANY-maze Video-Tracking Software. Statistical analysis was performed using the Prism GraphPad v.10.0 software.
Results. k18-hACE2-KI mice with expression of humanized *ACE2* gene under the control of the cytokeratin gene promoter showed a more pronounced ability to remember and retain the memory about the conditioned stimulus/context of the traumatic event in the PTSD-model when compared to C57Bl/6N mice. Anxiety measured in the light-dark box test was lower in k18-hACE2 mice than C57Bl/6N mice after FS. At the same time, there was a decrease in the open-field motor activity and there were no changes in spatial memory in the Barnes maze test. Lisinopril, an ACE inhibitor (28 days after FS), did not reduce traumatic memory in C57Bl/6N mice, indicating that the promnestic effect of *hACE2* gene expression is not a result of systemic hypotension and pointing at the involvement of the central mechanisms in the realization of *hACE2* gene effect in the pathological phenotype development.
Conclusions. The data indicate that the *hACE2* gene affects the stress response in mice. Specifically, the expression of *hACE2* gene in mice leads to increased memory of psychophysiological trauma and reduced extinction of traumatic memory compared to wild-type mice. This may be due to the modulation of the ACE2-dependent renin-angiotensin-aldosterone system in the brain. The decreased RAAS activity under the action of the ACE inhibitor lisinopril with a hypotensive effect did not affect memory in wild-type mice.

Keywords: post-traumatic stress disorder; *hACE2*; anxiety; memory; lisinopril

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ТРАНСГЕНЕЗ ПО ГЕНУ *ACE2* УСИЛИВАЕТ ПАМЯТЬ О ПСИХОФИЗИОЛОГИЧЕСКОЙ ТРАВМЕ В МОДЕЛИ ПОСТТРАВМАТИЧЕСКОГО СТРЕССОВОГО РАССТРОЙСТВА У МЫШЕЙ

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Введение. Развитие симптомов посттравматического стрессового расстройства (ПТСР) определяется комплексом факторов, которые не ограничиваются принадлежностью к классическим нейротрансмиттерным системам мозга или стрессовым гормонам. В частности, в механизмы ПТСР возможно вовлечение ренин-ангиотензин-альдостероновой системы мозга.
Цель. Изучение влияния экспрессии гена *hACE2* ангиотензинпревращающего фермента 2-го типа (ACE2) на тревожность и восприимчивость к психофизиологическому стрессу при моделировании ПТСР-подобного состояния у мышей, осуществленному с применением электрошока (ЭШ) конечностей.

Материалы и методы. Эксперименты были проведены на самцах мышей линий C57Bl/6N и k18-hACE2 возрастом 4–5 месяцев. Было сформировано три группы мышей линии C57Bl/6N: группа «контроль» (n = 7); группа «электрошок (ЭШ)» (n = 7); группа «ЭШ + лизиноприл» (n = 7); две группы мышей линии k18-hACE2: группа «контроль» (n = 7); группа «ЭШ» (n = 8). Проведено обусловливание реакции страха по Павлову с использованием ЭШ конечностей в качестве безусловного стимула. Мыши группы «ЭШ + лизиноприл» в течение 28 дней после психофизиологической травмы получали лизиноприл в дозе 10 мг/кг в сутки с питьевой водой. Оценку экспрессии реакции страха, отражающей память о психофизиологической травме, проводили на 7-е и 28-е сутки после воздействия ЭШ. Величину экспрессии реакции страха оценивали по относительному времени замирания. Для оценки общей локомоторной активности использовали тест «открытое поле». Оценку стратегии стресс-зависимого поведения изучали в тесте подвешивания за хвост; оценку тревожности — в тестах «светло-темная камера» и «приподнятый крестообразный лабиринт». Оценку пространственной навигации и динамики пространственного обучения проводили в тесте «лабиринт Барнса». Поведенческие параметры оценивали при помощи программного обеспечения ANY-maze Video-Tracking Software. Статистический анализ проведен с помощью пакета ПО Prism GraphPad 10.0.

Результаты. При моделировании ПТСР-подобного состояния с помощью ЭШ конечностей у мышей линии k18-hACE2 с экспрессией гена гуманизированного *ACE2* под контролем промотора гена цитокератина выявлена более выраженная способность, по сравнению с мышами линии C57Bl/6N, к запоминанию и удержанию памяти об условном стимуле/контексте травмирующего события. После воздействия ЭШ у мышей линии k18-hACE2 тревожность в тесте «светло-темная камера» была ниже по сравнению с мышами линии C57Bl/6N. При этом наблюдали снижение двигательной активности в тесте «открытое поле» и не обнаруживали изменений в пространственной памяти в тесте «лабиринт Барнса». Применение лизиноприла, ингибитора АСЕ, у мышей линии C57Bl/6N в течение 28 дней после ЭШ не приводило к снижению травматической памяти, что свидетельствует о том, что промнестический эффект экспрессии гена *hACE2* не является следствием системной гипотензии, и указывает на участие центральных механизмов в реализации эффекта гена *hACE2* при формировании патологического фенотипа.

Выводы. Полученные данные свидетельствуют о влиянии гена *hACE2* на формирование реакции на стресс у мышей, а именно, экспрессия *hACE2* у мышей сопровождается усилением памяти о психофизиологической травме и снижением экстинкции памяти о травме по сравнению с мышами дикого типа, что может определяться модуляцией активности ACE2-зависимого каскада ренин-ангиотензин-альдестероновой системы в мозге. Уменьшение регулирования активности РААС при применении ингибитора АСЕ лизиноприла с гипотензивным действием не оказывало влияния на память у мышей дикого типа.

Ключевые слова: посттравматическое стрессовое расстройство; *hACE2*; тревожность; память; лизиноприл

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INTRODUCTION

The significance of post-traumatic stress disorder (PTSD) among other stress-related diseases is determined by the high probability of health and life threats, the high incidence of subsequent mental disturbances, and the ineffectiveness of existing prophylactic and treatment means [1].

The recent discovery of previously unknown factors of maladaptive changes in the brain has intensified the search for approaches to overcoming the consequences of high-intensity acute stress and the problem of PTSD therapy ineffectiveness. For example, when studying COVID-19 outcomes, Hoffmann et al. [2] found that angiotensin-converting enzyme 2 (ACE2), which acts as the main receptor for the S1 subunit of the SARS-CoV-2 spike protein, can determine not only infectivity, but also may increase anxiety and lead to the development of depressive symptoms in the setting of viral infections [3].

ACE2 protein is an important element of the renin-angiotensin-aldosterone system (RAAS), whose components largely determine the systemic blood pressure. ACE2 degrades the pressor angiotensin II (AngII) and thus functionally balances the activity of the ACE-dependent pro-hypertensive RAAS cascade [4, 5]. According to Yang et al., mice with overexpression of the humanized *ACE2* gene (*hACE2*) under control of the cytokeratin k18 gene promoter can be considered as a model of viral infection of high neuroinvasiveness [6], which attributes a range of negative changes in the central nervous system [7]. However, upon overexpression of the *ACE2* gene or its pharmacological activation, the balance between AngII and its derivative with hypotensive activity — Ang1-7, may change in favor of the latter. Thus, Lima et al. and Meng et al. showed that reduced functional activity of the pro-hypertensive RAAS cascade in the absence of infection can have a positive effect on brain processes [8, 9].

Literature data also suggests that the local brain RAAS is involved in the mechanisms of specific activity of the nervous tissue [10]. In particular, under acute stress, the extracellular level of cathepsin D in the prefrontal

cortex increases [11]. Since cathepsin D is one of the endopeptidases that determine the conversion of angiotensinogen to AngI [12, 13], acute stress increases the likelihood of AngII formation from AngI and enhances the RAAS activity in the brain. It has also been shown that memory consolidation is impaired by the administration of AngII in the CA1 region of the hippocampus in the active avoidance test. This effect is mediated by angiotensin II type 1 receptor (AGTR1) and involves the ERK1/2 intracellular signaling cascade [14]. In turn, the reduction of characteristic anxiety in mice with total overexpression of *hACE2* is associated with an Ang1-7-mediated activation of Mas receptors and the related changes in the activity of GABAergic neurons in the basolateral amygdala [15, 16].

Assuming susceptibility to stress depending on the level of ACE2 expression or on its functional activity and/or the RAAS activity, it is of interest to study the stress-induced behavior of mice under conditions of *hACE2* gene expression [15] or under chronic administration of lisinopril, an ACE inhibitor, with permeability of the blood–brain barrier.

The aim of this study was to explore the effect of *hACE2* gene expression on anxiety and susceptibility to psychophysiological stress in k18-hACE2-KI mice in a foot electroshock (FS) model of PTSD.

MATERIALS AND METHODS

The experiments were conducted using male mice of the C57Bl/6N and k18-hACE2-KI lines (Andreevka Nursery of the Scientific Center for Biomedical Technologies and the Centre for Strategic Planning and Management of Biomedical Health Risks) aged 4–5 months. The animals were kept in cages with artificial ventilation, 5–7 animals per cage, at a temperature of 24°C and a 12-hour light/12-hour dark cycle (light on at 7:00 a.m. and light off at 7:00 p.m.). Water and standard feed were *ad libitum*.

Studies were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and approved by the Bioethics Commission of Centre for Strategic Planning and Management of Biomedical Health Risks of FMBA (Protocol No. 2 dated 15.02.2024).

Experimental design

For the study, the animals were randomly divided into groups of 7–8 individuals.

Three groups of C57Bl/6N mice were formed:

1. Control group (n = 7);
2. FS group (n = 7);
3. FS + lisinopril group (n = 7);

Two groups of k18-hACE2 mice:

4. Control group (n = 7);
5. FS group (n = 8).

Given that lisinopril was used for pharmacological modeling of possible hypotensive effects of *hACE2* expression, an additional comparison group of k18-hACE2 transgenic mice of the “FS + lisinopril” line was not introduced.

PTSD model. After habituation of mice to the housing conditions and to each other in the formed groups, a modeling of PTSD-like endophenotype was performed using the method of foot electroshock (FS), as described earlier in [17, 18]. Two or three days before the experiment, the animals were habituated for 3 min to a test plexiglass chamber (16×16×32 cm^3) placed in a soundproof box and equipped with an electrode grid floor connected to a DC generator and with a video camera (Fear Conditioning System, UgoBasil, Italy). During the test session, after a 1-min rest period, two 1.5 mA 2 s pulses were applied to the floor, one after the other, with a 1 min interval. After the second pulse, the animal was left in the chamber for an additional 1 min before being returned to its home cage. The mice from the control group remained in the FS chamber for 5 min.

Drug administration. Mice in the FS + lisinopril group received lisinopril at a dose of 10 mg/kg per day with drinking water for 28 days after psychophysiological trauma. The used dose of 10 mg/kg per day corresponds to the doses recommended for treatment of arterial hypertension in humans. Before the experiment, daily water consumption was monitored for a week to assess background water consumption and calculate the working concentrations of the lisinopril solution. The average daily fluid intake per animal of 4.46 mL/day accords with the data on water intake in adult mice known from numerous literature sources. Based on the preliminary assessment of water intake, a solution of lisinopril (Alsi Pharma, Russia) was prepared with the working concentration such that each animal received an average daily dose of 10 mg/kg of the drug. The lisinopril solution was renewed every other day. During the 28 days of therapeutic exposure, the daily water intake was assessed to monitor the received dose of the drug. However, minor deviations from the average consumption value could have an impact on the final effect severity, thus affecting the experiment outcome.

FS memory assessment. On days 7 and 28 after FS exposure, mice were placed in the test chamber for 3 min and the time of freezing (absence of any movements, except for those caused by the respiratory excursion of the chest for two or more seconds) was measured using the ANY-maze Video-Tracking Software. The magnitude of the fear response was expressed as a relative freezing time.

Behavior such as locomotor activity, anxiety, stress coping strategy, and spatial navigation/spatial learning were evaluated in a series of tests on days 29–32 after FS.

To assess the overall locomotor activity, the open field (OF) test was used. For this purpose, the animals were placed in the arena (41×41×33 cm^3) of the motor activity measurement system (Multiple Activity Cage, UgoBasil, Italy). The locomotor activity and verticalization (the total number of stands with support on the test arena walls and without support on the test arena walls) were assessed based on the total number of intersections between the beams of the photodetectors, which were located 2 cm apart on the panels on both sides of the arena at two horizontal levels. The test was conducted for 30 min under 300 lux.

The tail suspension test (TST) was used to evaluate the stress coping strategy. The immobility time was assessed by evaluating the freezing time in the first 3 min and second 3 min of the test separately.

The light/dark box test (LDB) was used to assess the anxiety level. The test was conducted in a chamber (42×40×40 cm^3) divided into equal-sized open and closed compartments (Light/Dark Box for Mice, UgoBasil, Italy). The open space avoidance behavior (latent period from the moment the experimental animal was placed in the center of the light compartment (LC) to the moment of the first entry into the dark compartment (DC), the number of entries into the compartments, the time spent in the compartments, and the total distance traveled) was assessed over a 10-min period. The illumination in the LC was 400 lux.

The elevated plus maze (EPM) test was also used to measure anxiety. The maze was located at a height of 60 cm from the floor and consisted of two open (OA) (80 cm×5 cm) and two closed (CA) (80 cm×5 cm) arms intersected at a right angle (Elevated Plus Maze for Mice, UgoBasil, Italy). The number of glances into the OA, the number of entries into the OA and CA, the time spent in the OA and CA, and the total distance traveled were evaluated over a 5-min period. The OA illumination was 400 lux. According to the test results, the anxiety index (AI) was calculated using the following formula: AI = 1 — [(time spent in the OA)/total test duration) + (number of entries to the OA/total number of entries to the arms)]/2 [17].

Spatial navigation and spatial learning dynamics were assessed in the Barnes maze test (BMT) using an open circular arena with a diameter of 100 cm (Barnes Maze for Mice, UgoBasil, Italy) with an illumination of the central area of 600–700 lux. The maze was divided into four segments, with a shelter placed under the surface of one of them. The training sessions (the first and second days of the test, “day 1” and “day 2”) and the test trial (the third day of the test, “test”) each lasted 3 min.

Behavior in the LDB, EPM, and BMT tests was analyzed using the Any-maze Video-Tracking Software, and the video was recorded using the DMK 22AUC03 video camera (IMAGINSOURCE, Germany) with the Computar A4Z2812CS-MPIR lens (Megapixel, China).

The experimental design is shown in Fig. 1.

Statistical analysis was performed using the PrismGraphPad v10.0+ software. The data are presented as the mean value ± the standard error of the mean value (M ± SEM). Given the sample sizes determined by the availability of transgenic animals, the normality of the data distribution was not assessed. However, all data were checked for outliers using an algorithm based on a nonlinear regression model [19]. A two-factor analysis of variance (ANOVA) was used to compare the estimated effects in the groups. When a significant influence of any main factor was detected, between-group comparisons were conducted using the Tukey test.

Depending on the parameter being evaluated and the specifics of the test, the following pairs of principal factors were established:

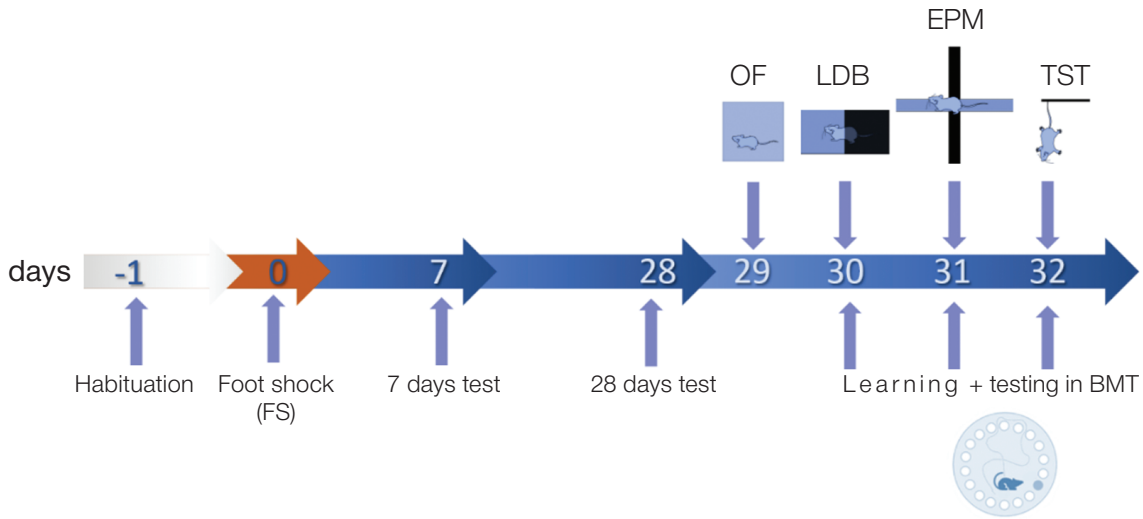

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Fig. 1. Design of experiment on modeling and phenotyping of PTSD-like state in mice: OF — open field test; LDB — light/dark box test; EPM — elevated plus maze; TST — tail suspension test; BMT — Barnes maze test

(1) FS (single FS exposure or no FS) × genotype (presence or absence of *hACE2*);

(2) genotype × time (minutes in the OF, days in the freezing assessment);

(3) FS × time (days in the freezing assessment and BMT parameters);

(4) groups (control, FS, or FS + lisinopril) × time (days in the freezing assessment and BMT and TST parameters).

In the case of assessing the lisinopril effect in all behavioral tests, except for assessing freezing and behavior in BMT and TST, a one-way analysis of variance was used to compare the groups ("no FS" *vs* "FS" *vs* "FS + lisinopril"). The differences were considered statistically significant at a p value of < 0.05.

RESULTS

Expression of *hACE2* gene was found to affect the behavioral activity of k18-hACE2-KI mice when compared to C57Bl/6N. A 1.2-fold decrease in the distance traveled in the OF was observed in intact k18-hACE2 mice compared to intact C57Bl/6N mice (genotype: $F(1.12) = 16.83$, $p = 0.0015$); the corresponding data are presented in Fig. 2a. In the LDB test, mice of the k18-hACE2-KI line showed a statistically significant 15.4-fold increase in the latent period before the first entry to the DC and a 1.9-fold increase in the time spent in the LC ($Fs > 7.98$, $p < 0.017$), which is confirmed by the results of post-hoc analysis ($p < 0.0205$); the corresponding data are presented in Figs. 2e and 2g.

The k18-hACE2 mice exposed to a single FS reacted more strongly to shock compared to wild-type mice (comparison of averages in the Student's t-test, $t = 3.561$, $df = 13$, $p = 0.004$). In addition, they remembered the conditioned stimulus/context of the traumatic event better, which was expressed as an increased freezing time after psychophysiological trauma in transgenic mice compared to C57Bl/6N mice on days 7 and 28 after FS exposure (genotype: ($F(1.12) = 226.98$, $p < 0.001$; time: ($F(1.12) = 24.79$, $p = 0.0003$; genotype × time: ($F(1.12) = 8.113$, $p = 0,015$). Moreover, these mice did not show signs of fear extinction (post-hoc: 7 days vs. 28 days; $p = 0.157$) (Fig. 3b), whereas fear expression in C57Bl/6N mice decreased over 28 days (post-hoc: 7 days vs. 28 days; $p = 0.0001$) (Fig. 3a).

Psychophysiological trauma caused delayed behavioral changes in k18-hACE2 mice, which differed from those observed in C57Bl/6N mice. The immobility time in the TST in k18-hACE2 mice was 2.4 times less than in C57Bl/6N mice in the first 3 min ($p = 0.049$) and 1.5 times less in the second 3 min ($p = 0.0137$) in the 6-min test (genotype: ($F(1.13) = 6.268$, $p = 0.029$) one month after FS; the corresponding data are shown in Fig. 2c.

Transgenic mice exposed to FS demonstrated a 14.4-fold increase in the latency period before the first entry into DC in the LDB test compared to wild-type mice (genotype: ($F(1.11) = 43.91$, $p < 0.001$; FS: ($F(1.12) = 9.201$, $p = 0.010$; genotype × FS: ($F(1.11) = 7.276$, $p = 0.021$) (Fig. 2e). In both experimental groups, a comparable decrease in the number of entries to the LC and DC after the FS was observed (FS: $F(1.13) = 3.134$, $p = 0.101$). However, only the transgenic mice showed a decrease in the time spent in the DC, which was not symmetrical to the increase in the time spent in the LC (FS: ($F(1.13) = 7.486$, $p = 0.017$; genotype: $Fs > 38.47$, $p < 0.001$; genotype × FS: ($F(1.11) = 7.704$, $p = 0.017$) (Figs. 2f, 2g).

The FS effect was generally not selective towards k18-hACE2-KI mice when assessing anxiety in EPM (FS: $Fs < 3.279$, $p > 0.097$, genotype × FS: $Fs < 2.137$, $p > 0.16$) (Fig. 2h, i, j), although a decrease in the distance traveled in the closed arms of the maze was found in k18-hACE2-KI mice, relative to that found in FS exposed C57Bl/6N mice (post-hoc: $p = 0.028$) (Fig. 2k). The average AI values for the C57Bl/6N control mice, for the C57Bl/6N mice that received FS, for the k18-hACE2-KI control mice, and for k18-hACE2-KI mice with FS were 0.933 ± 0.019, 0.954 ± 0.017, 0.996 ± 0.004, and 0.991 ± 0.008, respectively. Two-factor analysis of the AI variance revealed the genotype effect: $F(1.12) = 15.52$, $p = 0.002$; the FS effect: $F(1.13) = 0.345$, $p = 0.567$; the effect of factor interaction: $F(1.12) = 1.063$, $p = 0.323$. The subsequent post-hoc test indicated a statistically significant difference between control groups ($p = 0.005$).

Assessment of locomotor activity in the OF test revealed the genotype ($F(1.12) = 16.830$, $p = 0.002$) and FS ($F(1.13) = 5.810$, $p = 0.032$) effects of *hACE2* expression (Fig. 2a). In addition, a selective decrease in verticalization after FS was found in k18-hACE2 mice (genotype × FS: ($F(1.12) = 5.362$, $p = 0.039$ (post-hoc: $p = 0.007$); FS and genotype: $FS < 3.7495$, $p > 0.075$) (Fig. 2b).

Evaluation of spatial navigation/spatial learning in BMT showed that in C57Bl/6N mice, not in k18-hACE2 mice, the strategy of finding shelter after FS is optimized during three consecutive days of the test, which is expressed in an increase in the proportion of visited holes in the target segment (FS: ($F(1.12) = 2.150$, $p = 0.168$; day: ($F(1.12) = 4.434$, $p = 0.028$, FS × day: ($F(2.24) = 1.693$, $p = 0.205$ (post-hoc: day 1 compared to the third day ("test"), $p = 0.049$) (Fig. 2n). This was accompanied by a decrease in the time spent to find shelter (FS: ($F(1.12) = 1.011$, $p = 0.335$; day: ($F(1.12) = 6.561$, $p = 0.010$, FS × day: ($F(2.24) = 0.266$, $p = 0.768$ (post-hoc: day 1 compared to the third day ("test"), $p = 0.018$) (Fig. 2l). In terms of the proportion of entries to the shelter segment, the FS effect was not statistically significant ($Fs < 0.285$, $p > 0.466$) (Fig. 2m).

The lisinopril effect was separately analyzed in C57Bl/6N mice in a PTSD model. Lisinopril, when administered chronically orally (at a dose of 10 mg/kg per day), did not affect contextual memory in the PTSD model (post-hoc: 7 days $p = 0.609$ and 28 days $p = 0.341$) (Fig. 3a). The average AI values for mice of the

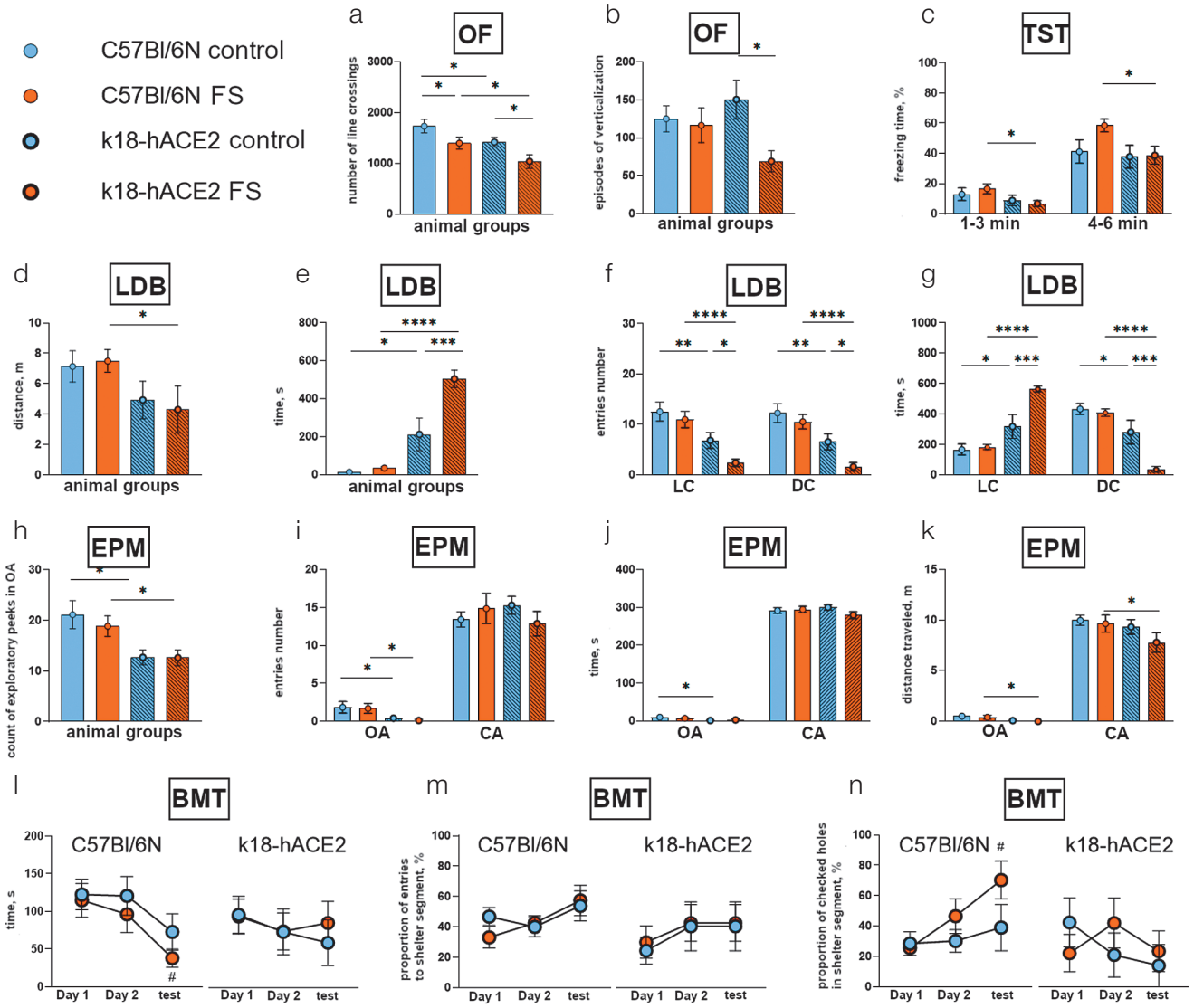

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Fig. 2. Comparison of the behavioral phenotype of C57Bl/6N and k18-hACE2 mice in the paradigm of assessing the delayed (29–32 days) effect of limbs electroshock (ES): a — locomotor activity; b — verticalization in open field (OF) test; c — freezing time in tail suspension test (TST); characteristic anxiety in light/dark box (LDB) test : d — total distance traveled in light compartment (LC) and dark compartment (DC); e — latent period before first visit to DC; f — number of entries to LC or DC; g — time spent in LC or DC; characteristic anxiety in elevated plus maze (EPM) test: h — count of exploratory peeks in open arm (OA); i — number of entries in OA or closed arm (CA); j — time spent in OA or CA; k — distance traveled in OA or CA; spatial navigation dynamics in Barnes maze test (BMT): l — time to find shelter; m– proportion of entries to shelter segment relative to number of entries to all segments; n — proportion of checked holes in shelter segment relative to total number of checked holes

Note: hatching — transgenic genotype; results of post-hoc comparison between groups: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; post-hoc comparison between day 1 and test when evaluating spatial navigation: # $p < 0.05$.

control group of the C57Bl/6N mice, for the C57Bl/6N mice who received FS, and mice of the C57Bl/6N line who received lisinopril after FS were 0.933 ± 0.019, 0.954 ± 0.017, and 0.927 ± 0.026, respectively, with the one-factor analysis of variance having revealed no differences between the groups F (2.18) = 0.446, $p = 0.647$.

No effect of lisinopril was found in the OF, TST, LDB, EPM, and BMT tests (Figs. 4a–j). There may be a possible effect on the dynamics of spatial navigation/spatial learning in LB; however, it was not confirmed by statistical analysis (post-hoc: $p > 0.073$) (Figs. 4h, 4i, 4j).

DISCUSSION

The obtained data indicate a complex profile of the *hACE2* gene expression effect on mouse behavior. On

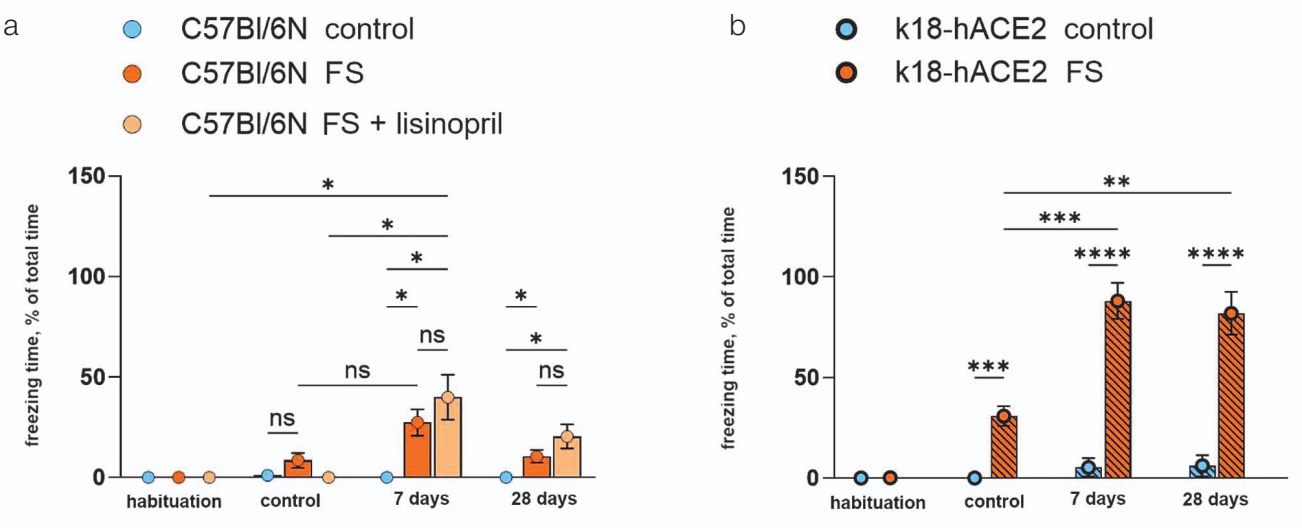

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Fig. 3. Freezing time dynamics in C57Bl/6N and k18-hACE2 mice: a — freezing time dynamics and effect of ACE inhibitor lisinopril (10 mg/kg per day) on the freezing time in C57Bl/6N mice; b — preservation of traumatic memory in k18-hACE2 mice

Note: hatching — transgenic genotype; post-hoc comparison results between groups: ns — not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; FS — foot shock.

the one hand, intact transgenic mice demonstrated an increase in resistance to an open illuminated space in the LDB test, which confirms the previously described anxiolytic effect of humanized *ACE2* gene overexpression in intact mice [15, 16]. On the other hand, our study established the effect of *hACE2* gene expression on the delayed consequences of PTSD-modeled acute stress. Thus, a longer exploration of LC, which is dangerous only potentially, in the LDB test and an increase in immobility, potentially of an adaptive nature, in the TST were observed. At the same time, the expression of the *hACE2* gene contributed to the strengthening and retention of traumatic memory, which was expressed in an increase in the freezing time and the absence of fear extinction (i.e., the inability to relearn the actual safety of the test chamber) within a month after psychophysiological trauma.

The enhancement of FS memory may be mediated by an increase in the FS perception since the immediate response of the transgenic mice to FS during conditioning was higher. It should be noted that this is likely to be determined by the perception mechanisms of the psychological component of stress rather than by an increase in the pain sensitivity, since a decrease in nociception was reported upon a decrease in efficiency of the ACE2-mediated signaling [20] and the transgenic model we used, on the contrary, involves an increase in the ACE2 function. Indeed, a comparison of our results with the characteristics of the behavioral endophenotype described in mice with *hACE2* gene overexpression [15, 16] indicates an increase in enzymatic activity of the ACE2 in k18-hACE2-KI mice, which may be mediated either by overexpression of the humanized gene or by the increased activity of *hACE2* compared to the wild type. The increase in enzymatic activity of the *hACE2* in k18-hACE2 mice may, in turn, lead to an increase in Ang1-7 production and an increase in the activity of the ACE2/Ang1-7-dependent RAAS cascade. The corresponding facilitation of MasR-dependent signaling in the mouse brain acts as a mechanism supporting neuroplasticity and enhancing memory [21, 22], as well as mediating anxiolytic and antidepressant action [23, 24].

Correa et al. and Fontes et al. considered RAAS regulators as potential targets in stress therapy [5, 25]. ACE inhibitors, AGTR1, as well as beta-blockers, have shown good results in the clinical setting of PTSD treatment. Preclinical studies revealed different effects. In the study by Marvar et al., the selective AGTR1 inhibitor losartan decreased traumatic memory, increased fear extinction, and did not affect the characteristic anxiety of the animals [26]. However, Braszko, Raghavendra et al. [27, 28] reported an increase in traumatic memory in a PTSD model. In our experiment, we studied the effect of ACE inhibitor lisinopril, an antihypertensive drug, on behavioral responses of C57Bl/6N mice in a PTSD model. We predicted that, at the selected dose of 10 mg/kg per day, which corresponds to the therapeutic doses used in clinical practice, lisinopril would exhibit an anxiolytic effect engaging its antihypertensive activity. However, chronic administration of lisinopril for 28 days did not affect either contextual memory or anxiety in mice. Earlier, Cohen et al. and Kao et al. observed no therapeutic effect of the

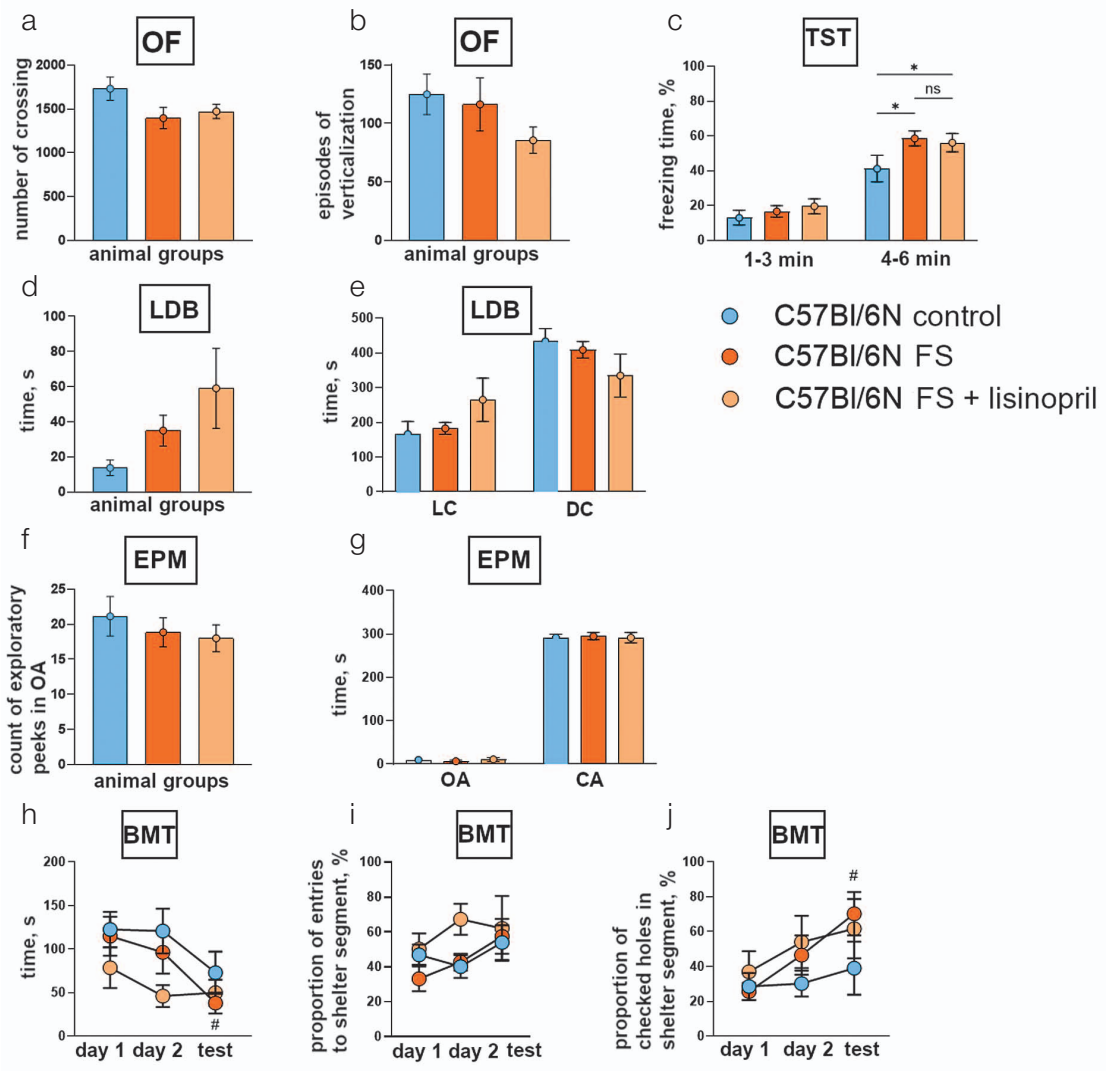

Figure prepared by the authors using their own data

Fig. 4. Effect of ACE inhibitor lisinopril on locomotor activity, anxiety, emotional coping, and spatial navigation in C57Bl/6N mice exposed to foot shock (FS): a — locomotor activity; b — verticalization in open field (OF) test; c — freezing time in tail suspension test (TST); d — latent period before the first entire to the dark compartment (DC); e — time spent in the light compartments (LC) and DC in the light/dark box (LDB) test; f — count of exploratory peeks in open arm (OA); g — time spent in open or closed arms (CA) in elevated plus maze (EPM) test; dynamics of spatial navigation in the Barnes maze test (BMT): h — time to find shelter; i — proportion of entries to shelter segment relative to number of entries to all segments; j — proportion of checked holes in shelter segment relative to total number of checked holes

Note: results of post-hoc comparison between groups: ns — not significant, $*p < 0.05$, $**p < 0.01$; results of a post-hoc comparison between data from day 1 and day 3 ("test") when evaluating spatial navigation: # $p < 0.05$.

beta-blocker propranolol in a PTSD model in mice [17, 29]. In combination, these data may point to the limited effect of normalizing systemic blood pressure with beta-blockers, as well as ACE inhibitors and AGTR1, in PTSD treatment. The ineffectiveness of lisinopril in our study may also be determined by the tertiary structure of the human enzyme. It is known that therapeutic effects of ACE inhibitors in PTSD depend on the *ACE* polymorphism, specifically determining resistance to therapy in the presence of the rs4311 TC nucleotide variant [30]. The probable increase in *ACE2* gene expression in response to chronic lisinopril use, which has already been described in the literature [31–35], was not sufficient to induce brain-specific ACE2/Ang1-7/MasR-dependent mechanisms.

CONCLUSION

The data obtained indicate the effect of the *hACE2* gene expression on mouse susceptibility to psychophysiological stress, which may be mediated by changes in the activity of the ACE2-dependent cascade of brain RAAS, and point to the significance of ACE2 in the traumatic memory acquisition in the PTSD model. Thus,

the *hACE2* expression in mice is accompanied by an increase in traumatic memory and by a decrease in extinction of traumatic memory compared to wild-type mice. The decreased RAAS activity under the action of the ACE inhibitor lisinopril with a hypotensive effect did not affect memory in wild-type mice.

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ANTIMICROBIAL EFFECT OF LOW-TEMPERATURE ARGON PLASMA ON SURGICAL INFECTION *IN VITRO*

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Introduction. Low-temperature argon plasma (LTAP) has been widely studied as an alternative approach to prevention of purulent infections in cases of reduced germicide effectiveness due to the developed pathogen resistance.
Objective. Survival assessment of opportunistic pathogens under the action of LTAP exposure in *in vitro* models.
Materials and methods. The study was carried out using ESKAPE clinical strains (a "superbug" group with a high epidemic potential for the formation of hospital strains) and reference strains from culture collections, as well as strain mixtures. A PLASMORAN plasma arc unit (Russia) was used as a LTAP source. One plasma generation mode, three distance variants (from the nozzle to the Petri dish culture plane — 10, 15, and 20 cm), four LTAP exposure options (15, 30, 45 s for bacteria and 30, 45, and 60 s for fungi) were used. Pathogens survival after LTAP exposure *in vitro* was assessed by bacterial growth inhibition.
Results. LTAP showed a significant antimicrobial action against clinical strains of ESKAPE Gram-negative bacteria *K. pneumoniae*, *R. aeruginosa*, *A. baumanii*, *E. coli*, Gram-positive bacteria MRSA and yeast-like fungi *C. albicans* under an exposure duration of 30–45 s for bacteria (the required dose of UV-A radiation is 37.8 J/m^2 UV-B 15.9 J/m^2 UV-C 34.2 J/m^2) and a distance of 10–15 cm from the plasma generator nozzle. The antimicrobial effect is manifested in the absence of pathogen growth on culture media at the site of LTAP exposure at a certain exposure dose, duration, and distance. This effect ensures a decrease in the titer of viable microorganisms not only in monocultures, but also in bacterial associations, both in reference strains from culture collections and in ESKAPE clinical strains.
Conclusions. PLASMORAN-generated LTAP exhibits significant antibacterial and antifungal effects with respect to both reference strains from culture collections and ESKAPE clinical strains. The efficacy of LTAP action ensures a decrease in the titer of viable microorganisms from 10^8–10^9 CFU to single CFU. The greatest effect of LTAP conditions (UV-A radiation dose of 37.8 J/m^2; UV-B 15.9 J/m^2; UV-C 34.2 J/m^2) on bacterial cultures is observed under a distance of 10–15 cm and an exposure duration of 30–45 s. The optimal conditions for fungi (UV-A radiation dose of 168.6 J/m^2; UV-B 68.4 J/m^2; UV-C 159 J/m^2) are a distance of 10 cm and an exposure duration of 60 s. Under standard dose, exposure duration, and distance, *A. baumannii* and *E. faecium* are more resistant to LTAP action than other studied bacteria. *C. albicans* is more resistant to LTAP action compared to bacteria, requiring a longer exposure and a shorter distance from the plasma generator nozzle to the treated surface. The results obtained require further study.

Keywords: low-temperature argon plasma; plasma flow; LTAP action; antimicrobial effect; *in vitro*; PLASMORAN plasma arc unit

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ИЗУЧЕНИЕ АНТИМИКРОБНОГО ДЕЙСТВИЯ НИЗКОТЕМПЕРАТУРНОЙ АРГОНОВОЙ ПЛАЗМЫ НА ХИРУРГИЧЕСКУЮ ИНФЕКЦИЮ В ЭКСПЕРИМЕНТЕ *IN VITRO*

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Введение. Применение метода на основе низкотемпературной аргоновой плазмы (НТАП) широко изучается в качестве альтернативного подхода к профилактике развития гнойных инфекций в случаях, когда эффективность антимикробных препаратов и антисептиков снижена из-за сформированной к ним устойчивости патогенов.
Цель. Оценка выживаемости условно-патогенных микроорганизмов, обладающих патогенным потенциалом, после воздействия факторов НТАП на модели *in vitro*.
Материалы и методы. Исследование выполнено с использованием клинических штаммов из группы ESKAPE-патогенов (группа супер-микроорганизмов с высоким эпидемическим потенциалом формирования госпитальных штаммов) и эталонных музейных культур, а также смеси штаммов. В качестве источника НТАП использовали плазменно-дуговую установку «ПЛАЗМОРАН» (Россия). В работе использовали один режим плазмогенерации, три варианта расстояния от среза сопла плазмотрона до плоскости расположения культуры в чашке Петри (10, 15 и 20 см), четыре варианта экспозиции действия факторов НТАП на культуры (15, 30, 45 с для бактерий и 30, 45 и 60 с для грибов). Оценку выживаемости патогенных микроорганизмов *in vitro* после воздействия НТАП определяли по задержке бактериального роста.
Результаты. Выявлено выраженное противомикробное действие в отношении клинических штаммов грамотрицательных бактерий из группы ESKAPE-патогенов *K. pneumoniae*, *P. aeruginosa*, *A. baumanii*, *E. coli*, грамположительных бактерий MRSA и дрожжеподобных грибов *C. albicans* при времени воздействия 30–45 с для бактерий (необходимая при этом доза излучения УФ-А 37,8 Дж/м^2, УФ-В 15,9 Дж/м^2, УФ-С 34,2 Дж/м^2) и расстоянии от сопла прибора 10–15 см. Противомикробное действие НТАП заключается в отсутствии роста микроорганизмов на питательных средах в месте воздействия НТАП при определенных дозе воздействия, времени экспозиции и расстоянии, обеспечивающих снижение титра жизнеспособных микроорганизмов не только монокультур, но и в ассоциации бактерий, как в отношении музейных эталонных культур, так и в отношении клинических штаммов из группы ESKAPE-патогенов.
Выводы. Факторы НТАП, формируемые плазменно-дуговой установкой «ПЛАЗМОРАН», обладают выраженным антибактериальным и противогрибковым действием как в отношении музейных эталонных культур, так и в отношении клинических штаммов рабочей коллекции группы ESKAPE-патогенов. Эффективность воздействия факторов НТАП обеспечивает снижение титра жизнеспособных микроорганизмов с 10^8–10^9 до единичных КОЕ. Факторы воздействия НТАП (доза излучения УФ-А 37,8 Дж/м^2; УФ-В 15,9 Дж/м^2; УФ-С 34,2 Дж/м^2) имеют наибольший эффект при обработке бактериальных культур на расстоянии 10–15 см с экспозицией 30–45 с; для грибов (доза излучения УФ-А 168,6 Дж/м^2; УФ-В 68,4 Дж/м^2; УФ-С 159 Дж/м^2) при расстоянии 10 см и времени воздействия 60 с. *A. baumannii* и *E. faecium* более устойчивы к факторам воздействия НТАП, чем другие исследованные бактерии при стандартных дозе, времени воздействия и расстоянии. *C. albicans* более устойчива к факторам воздействия НТАП по сравнению с бактериями, и их уничтожение требует большей экспозиции воздействия и меньшего расстояния от сопла плазмотрона до обрабатываемой поверхности. Полученные результаты требуют дальнейшего изучения.

Ключевые слова: низкотемпературная аргоновая плазма; плазменный поток; факторы НТАП; антимикробное действие; *in vitro*; плазменно-дуговая установка «ПЛАЗМОРАН»

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INTRODUCTION

Bacteria are ubiquitous inhabitants of the environment and the human body, which play a vital role in maintaining ecological balance. Among all known bacteria, including those without pathogenic potential, only about 1% can cause infections in patients with weakened immune systems. I. I. Mechnikov was the first to point out the possibility of normal human microflora to exhibit pathogenic properties. When skin damage occurs, both transient and resident opportunistic microflora can enter the wound surface. Opportunistic bacteria, such as *Staphylococcus aureus*, *Streptococcus sp.* and *Pseudomonas aeruginosa*, are common pathogens of skin and wound infections [1].

As a rule, antibacterial therapy is not aimed at infections caused by potentially pathogenic microflora due to widespread antimicrobial resistance [2–6]. Biofilms formed by attached bacteria further complicate the destruction of bacterial organisms [7–10]. In addition to the continuously extending list of drug-resistant microorganisms, antibacterial therapy is often inapplicable in patients with allergic and toxic drug reactions [11].

Growing antimicrobial resistance, which spreads globally, is a serious hazard to human health. In recent decades, the number of multidrug-resistant (MDR) bacteria isolated from the environment and biomaterials has increased, including Gram-negative bacteria *Escherichia coli*, *Serratia coli*, *Proteus mirabilis*, *Pneumocystis*

pneumoniae, *Pseudomonas aeruginosa*, and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) [12].

To date, research studies into infections caused by potentially pathogenic microflora have largely focused on identifying chemicals that are effective against bacteria. However, alternative approaches to the prevention and treatment of purulent infections based on physical factors are increasingly attracting attention, which is particularly relevant in view of the decreasing efficacy of germicides due to the phenomenon of antimicrobial resistance. Among them, low-temperature argon plasma (LTAP) is a promising local treatment tool with a significant bactericidal effect [13–16].

LTAP is a stream of partially ionized neutral gas produced at atmospheric pressure and macroscopic temperature [17]. The plasma stream contains charged particles (electrons, ions, free radicals, excited molecules) and ultraviolet radiation [18, 19]. The bactericidal effect of plasma is associated with the synergistic action of ionized elements and ultraviolet radiation, which leads to oxidative stress and bacterial DNA damage [20–23]. Therefore, LTAP has a significant antimicrobial potential.

Medical products and equipment are processed in three stages: disinfection, pre-sterilization cleaning, and sterilization. Reusable instruments, devices, and equipment are treated with mechanical, physical (thermal), chemical, or combined methods after every patient use. The mechanical method is based on wiping systems, when large-sized devices and instruments that do not come into contact with blood are treated with disinfectant wipes. The physical (thermal) method consists in heating in water at a temperature of 70–80°C, steam, or hot air (e.g., bed items are sterilized by steam under pressure; medical products made of glass, metals, and silicone rubber that are not contaminated with secretions are disinfected with 120°C air). The chemical method involves immersing the instruments in disinfectant containers. Physical and chemical disinfection methods can be used in combination, e.g., treating instruments in a cleaning solution simultaneously upon their heating. Non-thermal plasma can be effective for sterilizing medical equipment when thermal or chemical treatment is not suitable [13, 24, 25].

Low-temperature plasma has been reported to improve skin regeneration in cosmetology [26, 27]. It also showed efficacy in wound healing and pain reduction after hemorrhoidectomy, including due to its significant bactericidal effect [28]. LTAP treatment accelerated regenerative processes and wound cleansing during the correction of late purulent-inflammatory complications of contouring using polyacrylamide gel [29]. In [30, 31], the bactericidal effect of LTAP was observed for the first time, when treating contaminated dentin areas.

Due to its nonspecific and penetrating action, LTAP can reduce microbial contamination of tissues, ensuring local bactericidal effects on infected wounds and reducing wound inflammation. LTAP has a number of key advantages, including a high nonspecific bactericidal activity and a low potential for the development of resistance. In addition, LTAP is a promising method for treating acute and chronic wounds due to its relative simplicity, low cost, and a lack of special requirements for the treatment of wound surfaces.

This study is aimed at assessing the survival of opportunistic pathogens under the action of LTAP exposure in *in vitro* models.

MATERIALS AND METHODS

The study was carried out using the following ESKAPE clinical strains — *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, Gram-positive MRSA *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium* with multidrug resistance (according to the European Committee on Antimicrobial Susceptibility Testing — EUCAST), yeast-like fungi *Candida albicans*, reference strains from culture collections *E. coli* ATCC 10536, *K. pneumoniae* subsp. *pneumoniae* ATCC 700603, *Staphylococcus aureus* 906, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* 9/63, fungi *Candida albicans* ATCC 24433.

The strains were taken from the culture collection of the Laboratory of Microbiology and Parasitology (Centre for Strategic Planning of the Federal medical and biological agency). The study was conducted using certified reference materials with confirmed biochemical properties grown on dense agarized media (quality control). Reference cultures were acquired from national and state collections. The cultures were stored in a culture collection in a cryoprotective environment in low-temperature freezers at a temperature of –70°C.

The following dense agarized nutrient media were used for the microbial culture: Endo medium for the cultivation of *E. coli* culture, *K. pneumoniae*, *P. aeruginosa*; yolk-salt agar for the cultivation of *S. aureus*; Enterococcus-agar for the cultivation of *E. faecalis* and *E. faecium*; Sabouraud Fluid Medium for the cultivation of *C. albicans*.

In the study, the dynamic range of the action of LTAP conditions on the survival of individual ESKAPE pathogens strains and strain mixtures was assessed.

The research methodology was as follows. Daily cultures of microbial strains were drip-applied onto the surface of Petri dishes with agarized medium (d = 90 mm) to form a continuous lawn at the rate of 100 µL per dish. In the suspension applied to the agarized medium surface, each microbe content was 4.5 McFarland Standard No, which corresponds to 9.0×10^8–1.2×10^9 CFU/mL. The microbe suspension was thoroughly rubbed with a spatula over the entire agar surface. Then, each dish with the introduced microorganism was treated using a PLASMORAN plasma arc surgical wound treatment unit (TS 9444-001-43009282-2015 manufactured by Plasmoprom, Russia) in a preset mode, setting the

distance of 10, 15, and 20 cm (Factor L) and the exposure duration of 15, 30, and 45 s for bacteria and 30, 45, and 60 s for fungi (Factor T). In the study, one plasma generation mode, three options for the distance from the nozzle to the Petri dish culture plane, and four exposure options for the LTAP factors were used. To estimate the microbial titer used for inoculation in a bacterial suspension inoculum, a dilution method was used with inoculation on a Petri dish, followed by CFU growth counting on the agarized media surface.

The sectors of bacterial culture seeding within the exposure area, which was monitored by the illumination zone on the nutrient medium, were treated. Non-treated microbial crops were used as a control.

After the LTAP exposure, the culture dishes were placed in a thermostat for cultivation. The control group with microbial crops was also placed in a thermostat for further incubation.

The LTAP effect on microbial growth inhibition was assessed by the presence or absence of a lysis zone on the nutrient medium. The results were evaluated by measuring the diameter of the lysis zone delay and counting the number of grown clones of microorganisms in the lysis zone.

The culture dishes were incubated in a BD240 incubator (Binder, Germany) for 24–48 h at a temperature of (37 ± 1)°C for bacteria and at two temperature conditions (37 ± 1)°C and (26 ± 1)°C for fungi. After incubation, the grown colonies were counted in two ways: manually and using a Scan 1200 automatic colony counter (France). The purity of each culture was identified by mass spectrometry using a Microflex mass spectrometer with

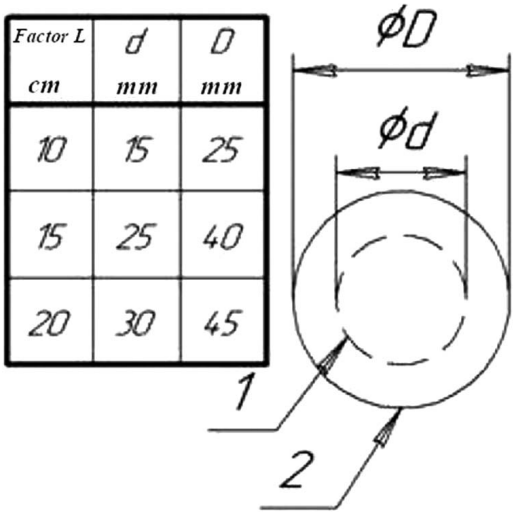


Figure prepared by the authors

Fig. 1. Geometry of the illumination spot on the treated surface formed by recombination radiation of LTAP depending on the value of Factor L: 1 — the border of the central and regional areas; 2 — the outer boundary of the edge area

specialized software (Bruker Daltonik GmbH, Germany). Subsequently, the biomass of the grown bacterial colonies in the exponential growth phase was removed from the surface of two dishes surface.

The LTAP effect on the survival of ESKAPE pathogens was evaluated after (24 ± 2) h for bacteria and after (48 ± 2) h and 7 days for fungi.

The plasma generator was operated in the following modes: the gas-dynamic flow of the working gas; recombination radiation with a wide spectrum — from the vacuum ultraviolet to the near infrared range; argon gas with significant catalytic properties in reactions involving oxygen, nitrogen, and their compounds; ozone formed as a result of recombination radiation action on atmospheric oxygen.

When treating the dishes with LTAP radiation, the dishes of the main group were positioned such that the LTAP radiation illumination spot of the marginal region covered the central inoculation area (Fig. 1). In the control groups of all the tested pathogens, the culture dishes were not exposed to LTAP radiation.

During exposure, the B2 device mode was used at the values of the time factor (T) of 15, 30, and 45 s and the distance factor (L) of 10, 15, and 20 cm. The energy characteristics used to illuminate the dishes are shown in Table 1. Since the device measures energy illumination (also known as radiation intensity) in W/m^2, the energy accumulation is expressed in J/m^2, which is equal to W/m^2 × radiation time (s). The LTAP radiation energy characteristics were measured by the manufacturer using a TKA-PKM 13 unit. The device is equipped with three sensors: UF-A (400–315 nm), UV-B (315–280 nm), and UV-B (280–200 nm).

The efficacy of LTAP exposure was assessed by calculating the percentage of bacterial and fungal death on the surface of control and experimental samples according to the formula:

$$\text{Efficacy (\%)} = 100 - ((\Sigma T \times 100)/C_m), \quad (1)$$

C_m — the mean number of bacteria in the initial suspension, equal to the ratio $\Sigma C/N$;
ΣC — the sum of all colonies grown on the dishes in all replications;
N — replication number;
T_m — the mean number of bacteria grown on the dishes after 24–48 h of crop incubation is equal to the ratio $\Sigma T/n$;
ΣT — the sum of all colonies grown on the dishes after 24–48 h of crops incubation in all replications;
n — the dish number (at least five for each test microorganism or a mixture of test microorganisms in accordance with EN ISO 11737-1[1]) used for the study (the same for control and LTAP-treated Petri dishes with test microbes).

The criterion of antimicrobial activity against test microorganisms or a mixture of test microorganisms was

[1] GOST ISO 11737-1-2012 Sterilization of medical products. Microbiological methods. Part 1. Evaluation of the microbial population on products.

Table 1. Characteristics of the PLASMORAN plasma arc surgical unit used for treatment

UV type	Factor T, s			
	15	30	45	60
Factor L = 10 cm				
UV-A	42.15	84.3	126.45	168.6
UV-B	17.1	34.2	51.3	68.4
UV-C	39.75	79.5	119.25	159
Factor L = 15 cm				
UV-A	18.9	37.8	56.7	75.6
UV-B	7.95	15.9	23.85	31.8
UV-C	17.1	34.2	51.3	68.4
Factor L = 20 cm				
UV-A	12.15	24.3	36.45	48.6
UV-B	4.68	9.36	14.04	18.72
UV-C	10.2	20.4	30.6	40.8

Table prepared by the authors using their own data

Note: radiation intensity measurement E (J/m^2) ± 10%

considered to be a decrease in colony growth by at least 99.99% or complete inhibition of their growth. Viable colonies were estimated by counting the grown colonies of each type of microorganism on the surface of nutrient media in Petri dishes (using a biological microscope Leica DM6B-Z, in/n-243302670221503917, s/n-503838 with an integrated camera; magn. ×100).

Statistical processing of the research results was carried out using the Statistica 10.0 software package (StatSoft Inc., USA). The standard deviation and error were calculated based on the results on each of five dishes, and the Student's *t*-test with the Bonferroni correction was applied, considering the distribution to be normal. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The first stage of the study was carried out using reference strains from culture collections in triplicate for each combination, including the distance and time of LTAP exposure.

When exposing reference strains from culture collections to LTAP (at different exposure distances and times), bacterial growth inhibition (efficacy 99.999–100%) was achieved against *E. faecalis* ATCC 29212, *E. faecium* 9/63, *K. pneumoniae* subsp. *pneumoniae* ATCC 700603, *E. coli* ATCC 10536, *P. aeruginosa* ATCC 10145, *S. aureus* 906; the corresponding data are presented in Table 2.

The effectiveness of bacterial growth inhibition at the level of 100% was achieved for *Enterococcus faecalis* at a distance of 10 cm after 15 s when exposed to a dose of UV-A 42.15 J/m^2, a dose of UV-B 17.1 J/m^2, and a dose of UV-C 39.75 J/m^2. At the same time, bacterial growth inhibition when exposed to doses of UV-A 84.3 J/m^2, UV-B 34.2 J/m^2, and UV-C 79.5 J/m^2 was achieved at an exposure duration of 30 s, and when exposed to UV-A 126.45 J/m^2, UV-B 51.3 J/m^2, and UV-C 119.25 J/m^2 after 45 s. When treated at a distance of 15 cm, bacterial growth inhibition was achieved after 45 s when exposed to UV-A 56.7 J/m^2, UV-B 23.85 J/m^2, and UV-C 51.3 J/m^2.

As a result of LTAP exposure of reference strains *Klebsiela pneumoniae* subsp. *pneumoniae* ATCC 700603, *E. coli* ATCC 10536, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* 9/63, *Pseudomonas aeruginosa* ATCC 10145 for 45 s with a UV-B dose of 15.9 J/m^2 and of *Candida albicans* 24433 ATCC for 60s with a dose of UV-B with 68.4 J/m^2 at a distance of 10 cm, all these microorganisms showed the absence of growth in the area of directed LTAP exposure; the corresponding data are shown in Fig. 2.

In the study, the LTAP effects on dishes with ESKAPE cultures were studied: Gram-positive bacteria MRSA *S. aureus*, *E. faecalis*, *E. faecium*; Gram-negative bacteria *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *E. coli*, as well as on yeast-like fungi C. albicans, using different exposure distances (from the plasma nozzle to the Petri

Table 2. Efficacy assessment of LTAP effect on the survival of reference strains from culture collections (%)

No.	Pathogen	LTAP exposure duration, s	Distances from the nozzle to the Petri dish culture plane, cm			Control, CFU/mL
			10	15	20	
			Effectiveness assessment (%)			
1	*Enterococcus faecalis* ATCC 29212	15	100	99.999	99.999	2×10^7
		30	100	99.999	99.999	
		45	100	100	99.999	
2	*Enterococcus faecium* 9/63	15	99.999	99.999	99.999	1.5×10^7
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
3	*Klebsiela pneumoniae* subsp. *pneumoniae* ATCC 700603	15	99.999	99.999	99.999	3.5×10^9
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
4	*Escherichia coli* ATCC 10536	15	99.999	99.999	99.999	3.0×10^9
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
5	*Pseudomonas aeruginosa* ATCC 10145	15	99.999	99.999	99.999	2.5×10^9
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
6	*Candida albicans* 24433 ATCC	15	99.999	99.995	99.95	1.2×10^7
		30	99.999	99.995	99.995	
		45	99.999	99.995	99.995	
7	*Staphylococcus aureus* 906	15	99.999	99.999	99.999	1×10^9
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	

Table prepared by the authors using their own data

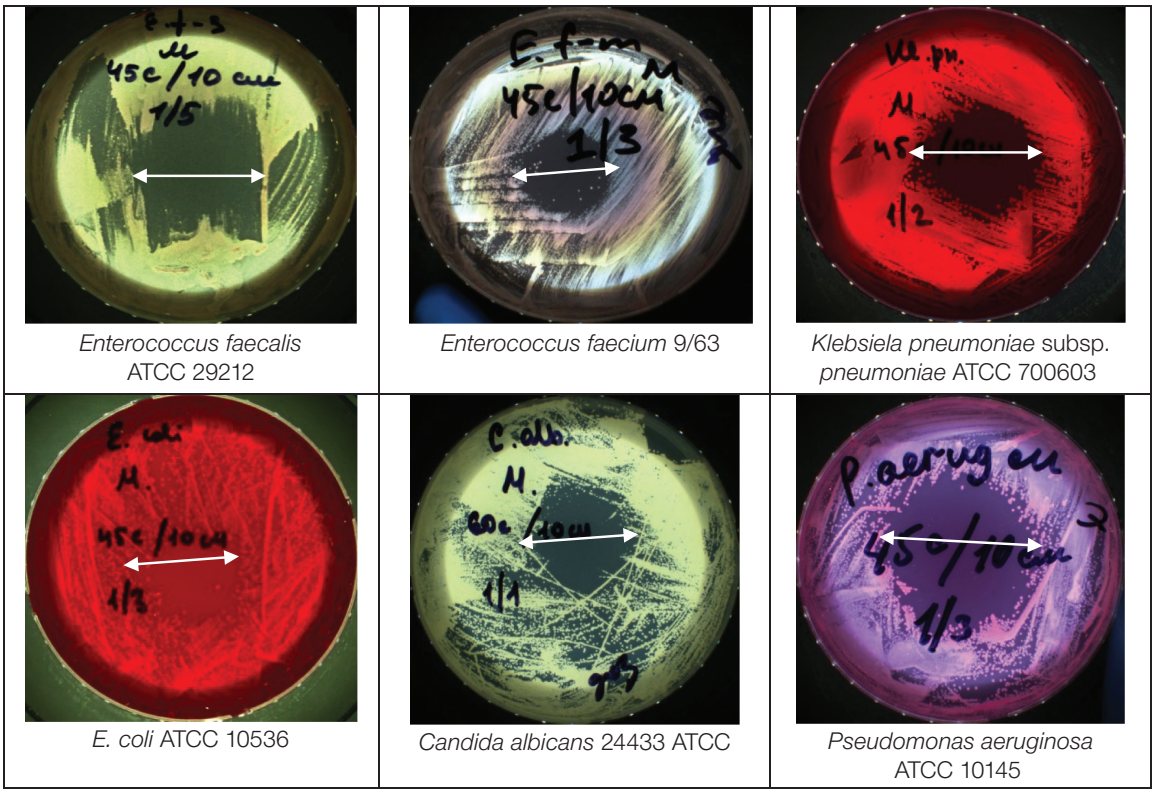
dish cultures plane) and exposure duration; the results are shown in Figure 3.

The research found that the use of variable LTAP parameters (distance and duration) allowed the efficacy of 99.999–100% in the inhibition of bacterial growth to be achieved (Table 3).

Experiments on LTAP action were carried out in five replications for ESKAPE clinical strains *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *E. coli*, Gram-positive bacteria MRSA *S. aureus*, *E. faecalis*, *E. faecium* with multiple drug resistance (according to the EUCAST), and in three replications for each combination of LTAP action on reference culture mixtures *E. coli* ATCC 10536, *K. pneumoniae* subsp. *pneumoniae* ATCC 700603, *S. aureus* 906, *P. aeruginosa* ATCC 10145, *E. faecalis* ATCC 29212, *E. faecium* 9/63.

When studying LTAP action on a mixture of reference strains from culture collections, the efficacy of 99.999% was achieved under variable parameters of distance and exposure duration. Survival was mainly observed for strains of *K. pneumoniae* subsp. *pneumoniae* ATCC 700603; viable colonies of *P. aeruginosa* ATCC 1014530, collection strains of *E. coli* ATCC 10536, *S. aureus* 906, *E. faecalis* ATCC 29212, *E. faecium* 9/63 were not detected (Table 4).

When evaluating LTAP effects on the survival of microbial clinical strain mixtures, a 99.997–99.999% efficacy was achieved compared with the initial inoculum



Enterococcus faecalis ATCC 29212 | *Enterococcus faecium* 9/63 | *Klebsiela pneumoniae* subsp. *pneumoniae* ATCC 700603

E. coli ATCC 10536 | *Candida albicans* 24433 ATCC | *Pseudomonas aeruginosa* ATCC 10145

Photos taken by the authors

Fig. 2. Efficacy assessment of LTAP effect on the survival of reference strains from culture collections: the distance from the nozzle to the Petri dish culture plane is 10 cm, LTAP exposure duration — 45 s (60 s for *Candida albicans* ATCC 24433); the arrow indicates the area of bacterial growth inhibition

level. After LTAP exposure, single colonies of clinical strains of *K. pneumoniae* and *A. baumannii* were found on the incubation dishes, while viable colonies of strains *P. aeruginosa*, *E. coli*, *S. aureus*, *E. faecalis*, *E. faecium*, *Candida albicans* were not found. The research results are presented in Table 5.

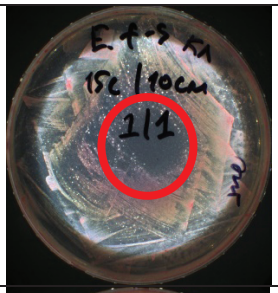
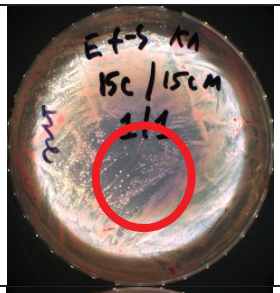
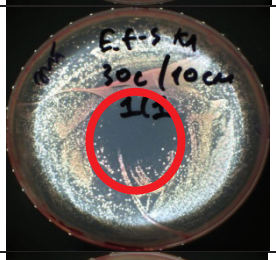
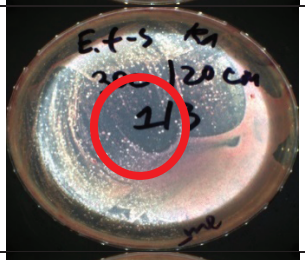
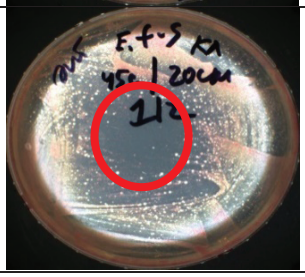
The antimicrobial action of LTAP is manifested in the death of bacterial cultures (Gram-positive and Gram-negative potentially pathogenic clinical strains of microorganisms with multiple drug resistance) and yeast fungi.

The study showed that 99.999% of microbial death occurs at the minimal exposure dose of UV-A 12.05 J/m^2, UV-B 4.68 J/m^2, UV-C 10.2 J/m^2 — combination of LTAP factors indicated in Table 5. An increase in the distance leads to a decrease in the LTAP effect. Conversely, an increase in the duration exposure intensifies the LTAP effect. Thus, an optimal combination of distance and duration enables the total LTAP energy to be transferred to the microorganisms. This observation was confirmed *in vitro* on microbial cultures such as *K. pneumoniae*, *A. baumannii*, and fungi *C. albicans*. Exposure at longer distances (15–20 cm) over time periods (15 s for bacteria and 30 s for fungi) had no effect on these cultures.

The LTAP exposure parameters that induced microbial death depended on the microbial type, exposure duration, and exposure distance. An exponential dependence of the viable bacteria number (*A. baumannii*, *K. pneumoniae*, and fungi *C. albicans*) on the energy illumination value (due to the exposure distance and exposure duration) at which the bacteria died, was found. It is important to note that LTAP treatment of MRSA *S. aureus* and *P. aeruginosa* disrupted the microbial viability completely at all values of distance and time parameters.

When studying the action of LTAP *in vitro* on the microbial survival of reference cultures of *E. coli* ATCC 10536, *K. pneumoniae* subsp. pneumoniae ATCC 700603, *S. aureus* 906, *P. aeruginosa* ATCC 10145, *E. faecalis* ATCC 29212, *E. faecium* 9/63, fungi *C. albicans* ATCC 24433 and ESKAPE strains *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *E. coli*, Gram-positive bacteria MRSA *S. aureus*, *E. faecalis*, *E. faecium*, with multiple drug resistance (according to the EUCAST), yeast-like fungi *C. albicans*, the PLASMORAN-generated LTAP ensured a decrease in the viable microbes titer from 10^8–10^9 to single CFU/mL, i.e., by 8–9 orders of magnitude, for both culture collection strains and clinical bacterial strains, with the efficacy ranging within 99.99–100%.

The literature has previously recognized the potential antibacterial effect of low-temperature plasma on ESKAPE pathogens [31]. In general, complete growth inhibition of unfixed ESKAPE pathogens when exposed to low-temperature plasma is achieved from 1 min to 3 h,

Exposure duration, s	Exposure distance, cm		
	10	15	20
Enterococcus faecalis			
15			
30			
45			
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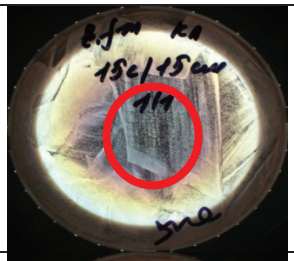
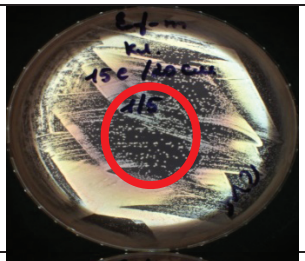
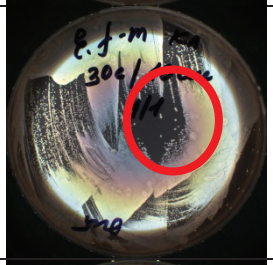
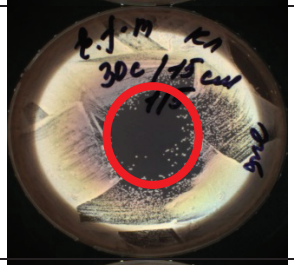
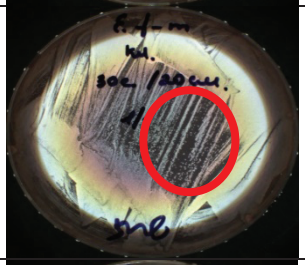
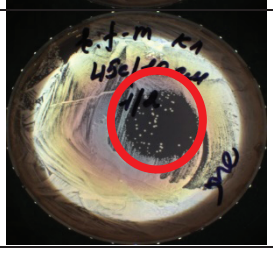
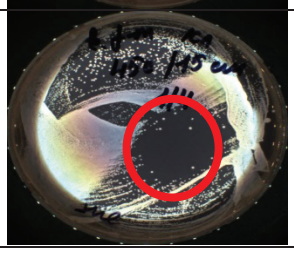
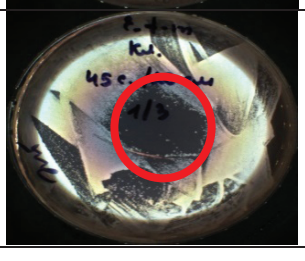
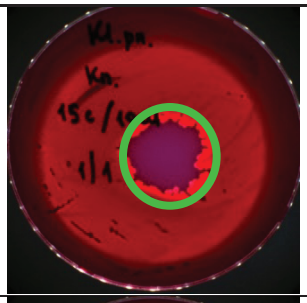
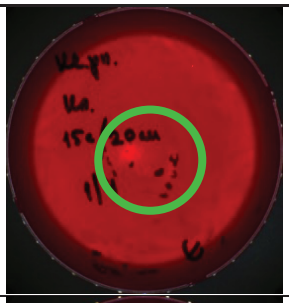
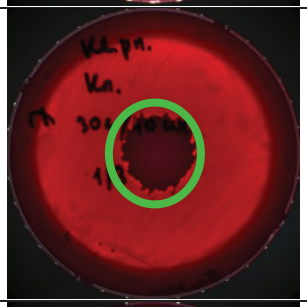
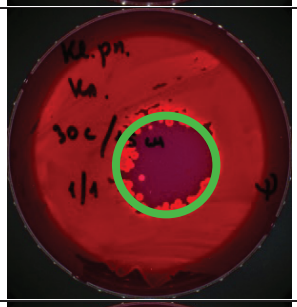
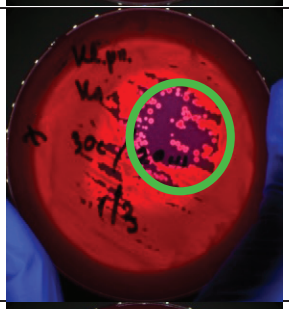
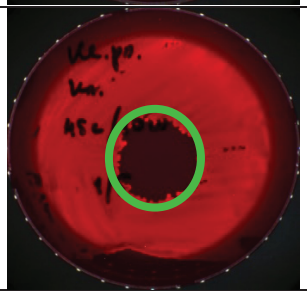
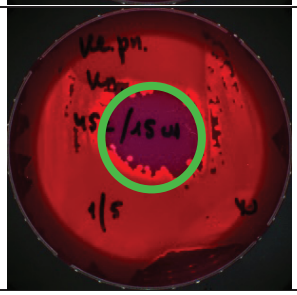
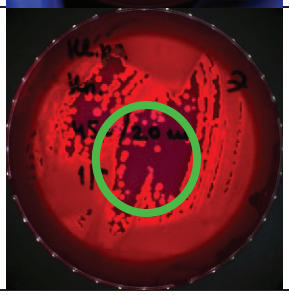
15			
30			
45			

Photos taken by the authors

Fig. 3. Efficacy of LTAP action on the survival of ESKAPE clinical strains: the area of bacterial growth inhibition is indicated; magn. ×100

Fig. 3 (continued)

Exposure duration, s	Exposure distance, cm		
	10	15	20
K. pneumaniae			
15			
30			
45			
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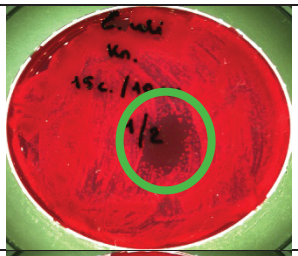
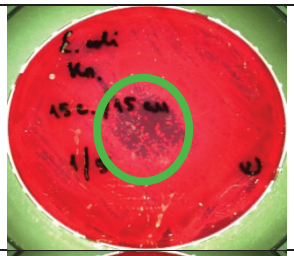
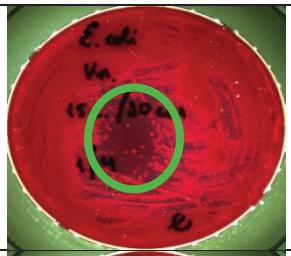
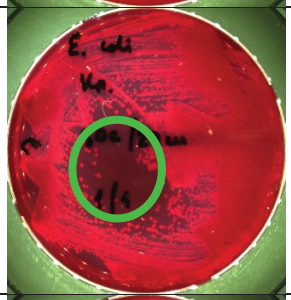
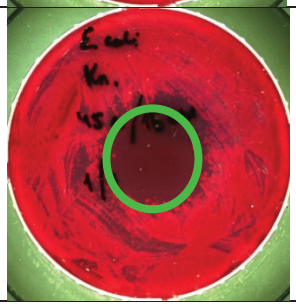
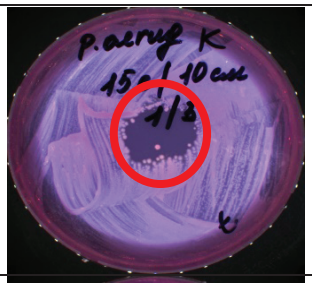
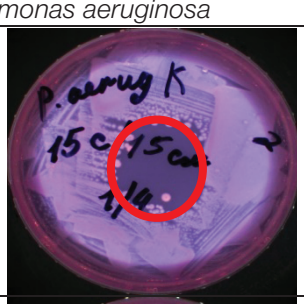
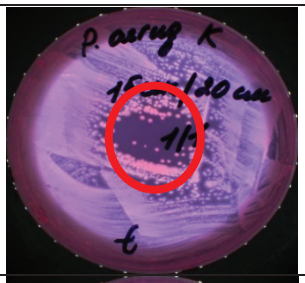
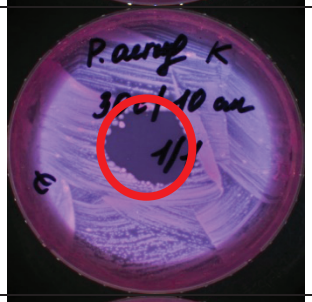
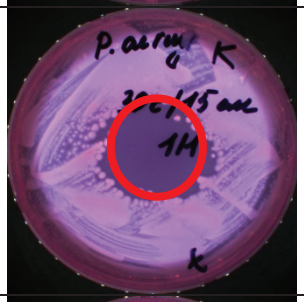
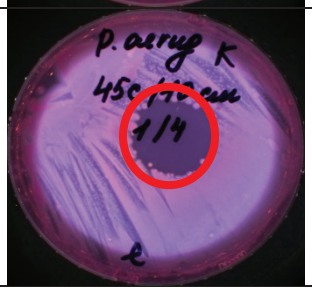
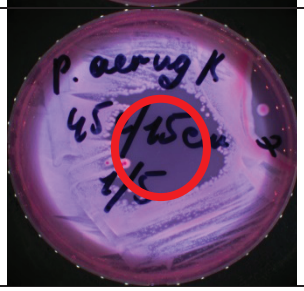
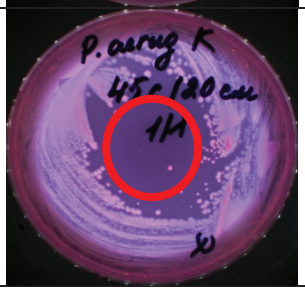
15			
30			
45			

Fig. 3 (continued)

Exposure duration, s	Exposure distance, cm		
	10	15	20
Pseudomonas aeruginosa			
15			
30			
45			
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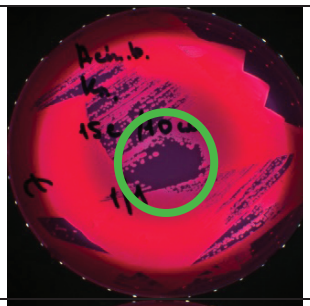
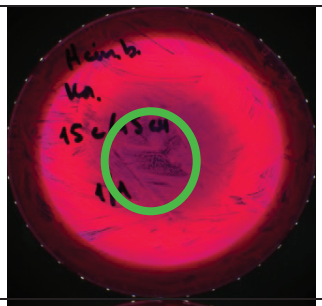
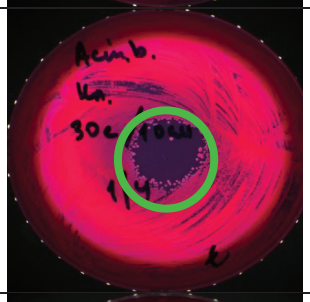
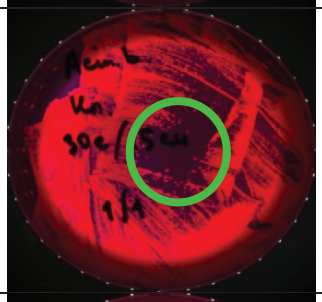
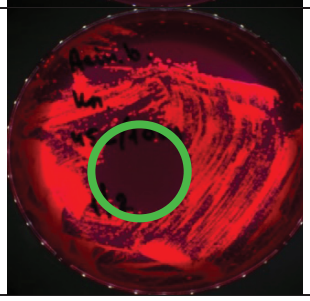
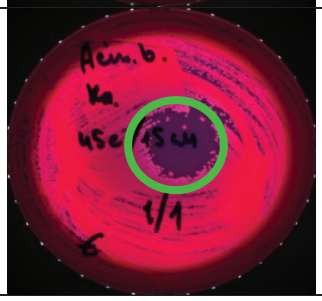
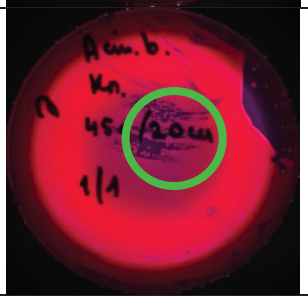
15			
30			
45			

Fig. 3 (continued)

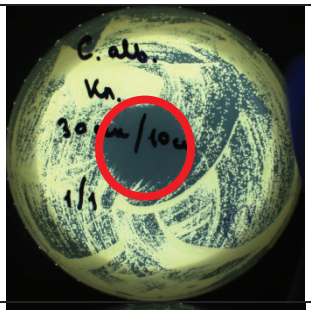
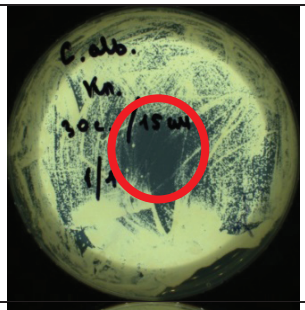
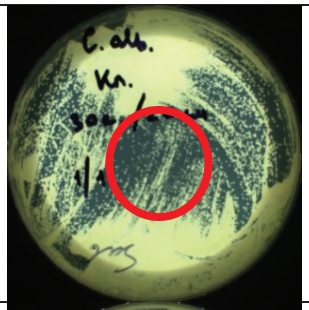
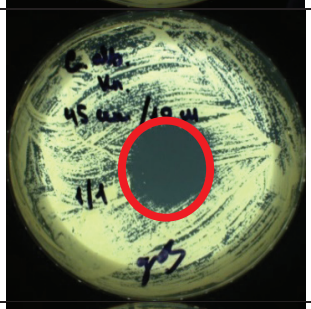
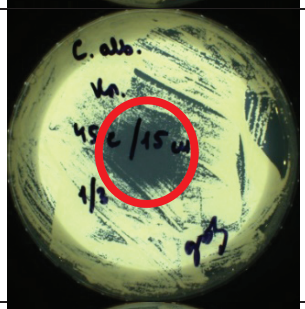
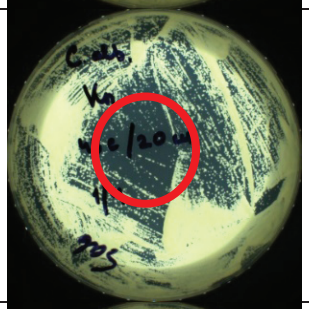
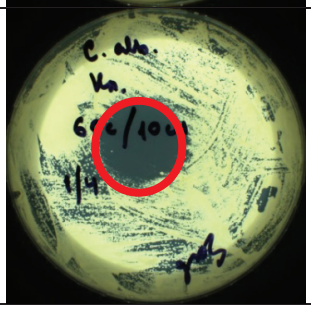
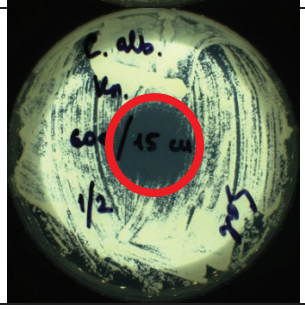
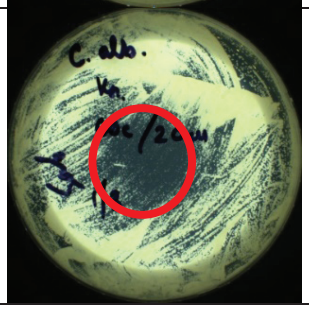
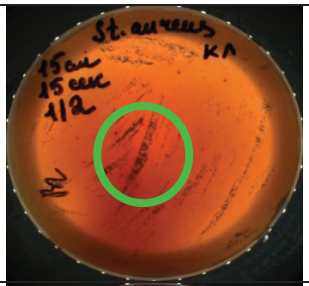
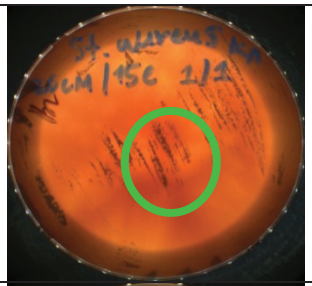
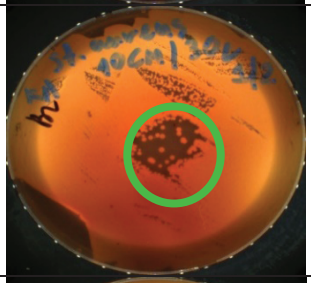
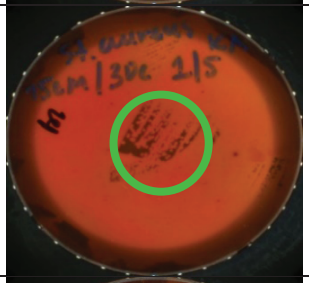
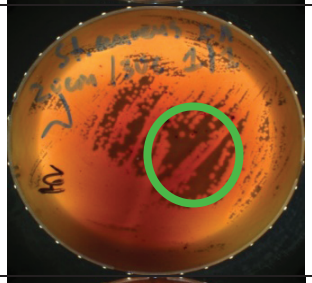
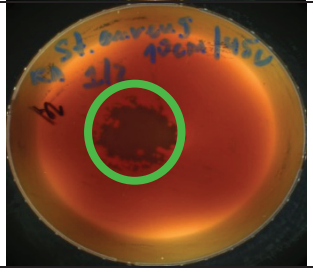
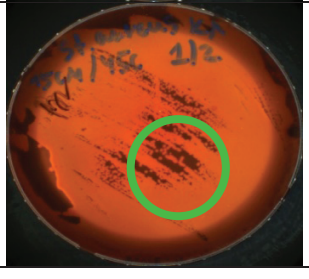
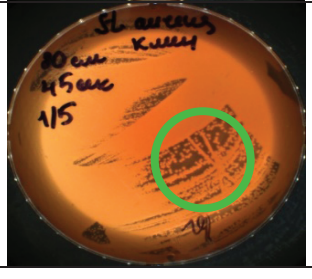
Exposure duration, s	Exposure distance, cm		
	10	15	20
Candida albicans			
30			
45			
60			
Staphylococcus aureus			
15			
30			
45			

Table 3. Efficacy assessment of LTAP action on the survival of microbial strains

No.	Pathogen	LTAP exposure duration, s	Distance from the nozzle to the Petri dish culture plane, cm			Control, CFU/mL
			10	15	20	
			Effectiveness assessment (%)			
1	*Enterococcus faecalis*	15	99.999	99.999	99.999	1.4×10^8
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
2	*Enterococcus faecium*	15	99.999	99.999	99.99	4.0×10^8
		30	99.999	99.999	99.99	
		45	99.999	99.999	99.999	
3	*Klebsiela pneumoniae*	15	99.999	99.999	99.999	5.4×10^8
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
4	*Escherichia coli*	15	99.999	99.999	99.999	4.0×10^8
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
5	*Pseudomonas aeruginosa*	15	99.999	99.999	99.999	3.3×10^8
		30	99.999	99.999	99.999	
		45	99.999	99.999	99.999	
6	*Acinetobacter baumanii*	15	99.999	99.99	99.99	1.5×10^8
		30	99.999	99.999	99.99	
		45	99.999	99.995	99.99	
7	*Candida albicans* *Staphylococcus aureus*	15	100	99.99	99.99	1.0×10^7
		30	99.999	99.999	99.99	
		45	99.999	99.999	99.99	
8	*Staphylococcus aureus*	15	99.999	99.998	–	2.1×10^8
		30	99.999	99.998	–	
		45	99.999	99.998	–	

Table prepared by the authors using their own data

depending on the device, type of discharge, output voltage, and other factors [31]. The most rapid growth inhibition of ESKAPE pathogens was described by Flynn et al. (2015) [32]. When using thermal plasma of atmospheric pressure, a rapid antibacterial effect was found against all ESKAPE pathogens in an unfixed (planktonic) growth form. Complete destruction of *Enterobacter cloacae* was achieved in 45 s, *P. aeruginosa* — in 60 s, *E. faecium*, *K. pneumoniae* and *A. baumannii* were completely inactivated within 120 s. *S. aureus* was the most stable, requiring a 240 s exposure to low-temperature plasma for its inactivation.

As a research result, a high efficacy of LTAP in experimental conditions was achieved with the proportion assessment of death of viable bacterial and fungal cultures. In the present study, almost complete destruction of viable cells of ESKAPE pathogen test cultures occurred during the LTAP exposure of 15 s, at a dose of UV-A 12.15 J/m^2, UV-B 4.68 J/m^2, and UV-C 10.2 J/m^2 at a distance of 20 cm.

Table 4. Efficacy assessment of LTAP action on the survival of reference strains from culture collections

Exposure duration, s	Exposure distance, cm			Control, CFU/mL
	10	15	20	
	Efficacy, %			
15	99.999	99.999	99.999	1×10^9
30	99.999	99.999	99.999	
45	99.999	99.999	99.999	

Table prepared by the authors using their own data

Table 5. Efficacy assessment of LTAP action on the survival of a mixture of microbial strains

Exposure duration, s	Exposure distance, cm			Control, CFU/mL
	10	15	20	
	Efficacy (%)			
15	99.999	99.997	99.997	4×10^8
30	99.999	99.997	99.997	
45	99.999	99.997	99.997	

Table prepared by the authors using their own data

C. albicans yeast-like fungi were found to be more resistant to LTAP exposure compared to bacteria. Their destruction requires a longer exposure and a shorter distance from the nozzle to the treated surface, while the exposure efficacy is similar to that for bacteria, i.e., 99.99–100%.

A number of studies have shown that fungi are less sensitive to low-temperature plasma than bacteria [33]. However, the effect of low-temperature plasma in inactivating fungi depends on the fungus type, the gases supplied, the distance between the plasma device and the sample, and exposure duration [34, 35]. Most studies have reported that non-thermal and low-temperature plasma can effectively inactivate *Candida albicans* yeast cells [34].

Some researchers emphasize that the exposure of strains to low-temperature plasma is associated with safety problems; namely, microorganisms that survive plasma exposure can be modified both genetically and phenotypically, representing danger to the environment. Thus, *C. albicans* that survived plasma treatment demonstrated genetic variability, although showing no significant changes in metabolism and drug susceptibility [35]. On the one hand, this indicates that low-temperature plasma treatment is associated with a low probability of the formation of genetically and phenotypically unfavorable strains [34, 35]; on the other, more experimental data is needed to solve the safety problem. Further studies in this direction should be conducted to assess the safety of LTAP exposure and the contribution of its main parameters to the antibacterial and proliferative effect for the treatment of postoperative wounds in *in vivo* models.

CONCLUSIONS

1. PLASMORAN-generated LTAP was found to exhibit significant antibacterial and antifungal effects both against reference strains from culture collections and against ESKAPE clinical strains: *K. pneumoniae*, *R. aeruginosa*, *A. baumannii*, *E. coli*, Gram-positive bacteria MRSA *S. aureus* and *E. faecalis*, with multiple drug resistance (according to the EUCAST system), and yeast-like fungi *C. albicans*. The efficacy of LTAP exposure is manifested in the decreased titer of viable microorganisms from 10^8–10^9 CFU to single CFU, i.e., by 8–9 orders of magnitude for bacteria, ranging within 99.999–100%.

2. *A. baumannii and E. faecium* are more resistant to LTAP exposure than other bacteria studied.

3. *C. albicans* is more resistant to LTAP exposure compared to bacteria, and their destruction requires a longer exposure duration and a shorter distance from the plasma nozzle to the treated surface, with an impact efficiency ranging 99.99–100%.

4. The greatest effect of LTAP exposure is achieved when treating potentially pathogenic microorganisms at a distance of 10–15 cm and an exposure duration of 30–45 s for bacteria (the required dose of UV radiation is

37.8 J/m^2 UV-B 15.9 J/m^2 UV-C 34.2 J/m^2) and at a distance of 10 cm and exposure time 60 s for fungi (the required radiation dose is UV-A 168.6 J/m^2 UV-B 68.4 J/m^2 UV-C 159 J/m^2).

5. Exposure to PLASMORAN-generated LTAP ensures a decrease in the viable microbial titer not only in monospecies culture, but also in the bacterial association both in relation to museum reference cultures and in relation to ESKAPE clinical strains *K. pneumoniae*, *R. aeruginosa*, *A. baumannii*, *E. coli*, Gram-positive bacteria MRSA *S. aureus* and *E. faecalis*, which have multidrug resistance.

6. The use of LTAP for treating wounds is of great relevance for the prevention of purulent complications in surgical departments, including at the stages of evacuation of the wounded during military operations.

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PSYCHOPHYSIOLOGICAL TRAINING OF PILOTS FOR CASES OF EMERGENCY COCKPIT DECOMPRESSION

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Introduction. In the stratosphere, when the aircraft cockpit is depressurized, the pilot switches to breathing pressurized oxygen. However, breathing under such conditions leads to the development of adverse processes that affect the functional state of the body and reduce the quality of aircraft piloting. Programs of psychophysiological training of pilots for such conditions include breathing and speech training under oxygen overpressure.
Objective. Effectiveness assessment of a five-day breathing and speech training course under oxygen overpressure in Vietnamese test subjects.
Materials and methods. The study involved 35 Vietnamese test subjects aged 19–32. The assessment of the development of breathing skills under oxygen overpressure (OOP) was based on the dynamics of psychological parameters and the pronunciation accuracy of control words. The study used a KM-35 demand oxygen mask in combination with a ZSh-7A pilot protective helmet and a VKK-15 altitude compensating suit to create counterpressure on the chest. OOP was created using the BARS-GD hardware and software complex. The developed course of breathing and speech training under OOP consists of five OOP breathing sessions, which are conducted once a day during five consecutive days. Each session involves breathing under OOP in a sequential and continuous manner at five stages with a breathing time of 2 min at each stage. OOP was created at levels ranging 150–1000 mmHg. The functioning of the central nervous system (CNS) was assessed based on the average time of simple and complex visual-motor reactions (SVMR, CVMR) and the response to a moving object (RMO). The level of situational anxiety, well-being, activity, and mood was assessed using the wellbeing, activity, mood (WAM) questionnaire. Statistical analysis was performed using the SPSS 26 software.
Results. As a result of the five-day training course, a statistically significant decrease in the pre-stress level of situational anxiety by 3.9% was observed. Prior to the training course, in the setting of simulated rapid cockpit decompression, a decrease in well-being and mood indicators by 3.7% and 5.7%, respectively, was noted. In addition, the experiment recorded an increase in psychophysiological reserves, which was confirmed by statistically significant changes in the time of simple and complex visual-motor reactions, as well as the results of testing the response to a moving object before and after the training course.
Conclusions. The data obtained confirmed the effectiveness of the developed five-day training course, as a result of which the test subjects increased their psychological and psychophysiological readiness to perform tasks under conditions of a sharp decrease in pressure in the pressurized cabin of an aircraft and the operation of high-altitude equipment. The developed five-day training regime of Vietnamese military personnel is recommended for integration into the training system of pilots for high-altitude and stratospheric flights.

Keywords: stratospheric flight; cockpit decompression; flight safety; professional reliability; Vietnamese pilot; emergency situation; psychophysiological readiness

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ПСИХОФИЗИОЛОГИЧЕСКАЯ ПОДГОТОВКА ЛЕТЧИКОВ К ДЕЯТЕЛЬНОСТИ В УСЛОВИЯХ АВАРИЙНОЙ РАЗГЕРМЕТИЗАЦИИ КАБИНЫ САМОЛЕТА

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Введение. В условиях стратосферы при разгерметизации кабины самолета летчик переходит в режим дыхания кислородом под избыточным давлением. Однако дыхание в таких условиях приводит к развитию неблагоприятных процессов, влияющих на функциональное состояние организма и снижающих качество пилотирования самолета. В качестве психофизиологической подготовки летчиков к работе в таких условиях предусмотрена тренировка дыхания и речи под избыточным давлением кислорода.
Цель исследования. Оценка эффективности 5-дневного курса тренировки дыхания и речи под избыточным давлением кислорода у вьетнамских испытателей.

Материалы и методы. В исследовании приняли участи 35 вьетнамских испытателей в возрасте 19–32 лет. Оценка сформированности навыка дыхания под избыточным давлением кислорода (ИДК) осуществлялась по динамике психологических показателей, психофизиологических резервов четкости произношения контрольных слов. В исследовании использовали кислородную маску КМ-35 в комплекте с защитным шлемом ЗШ-7А; для создания контрдавления на грудную клетку использовался высотно-компенсирующий костюм ВКК-15. Создание ИДК осуществлялось на аппаратно-программном комплексе БАРС-ГД. Разработанный курс тренировки дыхания и речи под ИДК представляет собой пять сеансов дыхания ИДК, которые проводятся один раз в день в течение пяти дней подряд. Каждый сеанс представлял собой дыхание под ИДК последовательно и непрерывно на пяти ступенях с созданием избыточного давления 150–1000 мм вод. ст., время дыхания на каждой ступени составляло 2 мин. Оценка уровня функционирования центральной нервной системы (ЦНС) проведена по среднему времени простой и сложной зрительно-моторной реакции (ПЗМР, СЗМР) и реакции на движущийся объект (РДО). Уровень ситуативной тревожности, самочувствия, активности и настроения оценивали по методике САН. Статистический анализ проведен с использованием пакета прикладных программ SPSS 26.
Результаты. В результате 5-дневного курса тренировки отмечено статистически значимое снижение донагрузочного уровня ситуативной тревожности на 3,9%. До курса тренировки после моделирования быстрой разгерметизации кабины самолета отмечали снижение показателей самочувствия и настроения на 3,7 и 5,7% соответственно. Также зарегистрировано повышение психофизиологических резервов, что подтверждено статистически значимым изменением времени простой и сложной зрительно-моторной реакции, а также результатов теста — реакцией на движущийся объект до и после курса тренировки.
Выводы. Полученные данные показали эффективность разработанного 5-дневного курса тренировки, в результате которого у испытателей сформировалась психологическая и психофизиологического готовность к выполнению задач в условиях резкого снижения давления в гермокабине летательного аппарата и работы высотного снаряжения. 5-дневный режим тренировки вьетнамских военнослужащих целесообразно интегрировать в систему подготовки летчиков к высотным и стратосферным полетам.

Ключевые слова: стратосферный полет; разгерметизация кабины; безопасность полетов; профессиональная надежность; вьетнамский летчик; аварийная ситуация; психофизиологическая готовность

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование одобрено на заседании независимого этического комитета при Военно-медицинской академии им. С.М. Кирова (протокол № 274 от 24.01.2023). Все участники подписали добровольное информированное согласие на участие в исследовании.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The current intensive development of aviation science and technology is associated with an increased emotional, physical, informational, and mental stress experienced by the pilot–aircraft–environment system. In this system, the pilot becomes an increasingly vulnerable element [1] due to the physiological and psychophysiological limits of the human being as a biological object [2].

Aviation medicine and flight physiology aim to preserve the professional health of pilots in the face of rapid technological advancements. This goal is achieved through technical solutions that compensate for the extreme factors of aviation flight and complex missions, as well as by continuously enhancing the professional reliability of pilots by improving their ability to perform tasks in extreme and emergency situations [3, 4]. The psychophysiological training of pilots for flights is carried out using a diverse set of ground-based training exercises and special equipment [5–8].

One of the key methods for maintaining the professional reliability of pilots for flights in the conditions of cockpit decompression in the stratosphere involves breathing training under overpressure of oxygen (OOP). The pilot must be trained to enhance the ability to withstand overpressure[1]. This is a prerequisite for the admission of a pilot to stratospheric flights. Breathing and speech training under OOP allows pilots to learn to breathe and maintain radio communications in the event of an emergency (in the case of cockpit decompression in the stratosphere) [9]. This is an important difference between military pilots and civil aviation pilots, who perform flights below the stratosphere and do not need such training.

Pilot training in breathing and speech under OOP is carried out regularly in order to form new biomechanical patterns of breathing [10]. To that end, an oxygen training device can be used to create excess gas pressure in the mask and protective equipment in ground conditions. Pilot training can also be conducted under conditions of high atmospheric rarefaction during barometric ascents to an altitude of more than 12 km [11]. Training is performed on an aviation simulator. The pilot must develop stable piloting skills and psychophysiological readiness to perform correct actions and control the aircraft in the event of a cockpit decompression at high altitudes [12, 13].

Currently, the Vietnam Air Force is equipped with modern aircrafts capable of high-altitude and stratospheric

[1] Gradwell DP. Human physiological responses to positive pressure breathing for high altitude protection. PhD thesis. London: University of London; 1993.

flights [14], which are becoming increasingly important in today's world. However, the incomplete system of psychophysiological training (lack of training in breathing and speech under OOP, limited use of special equipment for checking the fit of high-altitude equipment, particularly in hot and humid climates) poses a significant obstacle for Vietnamese pilots to perform stratospheric flights. This determines the need to develop a rational breathing and speech training regimen using modern and innovative equipment [10]. The developed regimen should include both an introductory component, which is performed at a low level of OOP, and a training component, which allows the test pilot to adopt specific biomechanics of breathing with a short passive inhalation and a long active exhalation, as well as to develop the skill of pronouncing the words necessary for radio communication [7]. This will enhance the professional reliability of pilots not only in emergency situations but also during high-g maneuvers.

In this study, we set out to assess the development of breathing and speech skills under oxygen overpressure in Vietnamese test subjects after completing a training course.

MATERIALS AND METHODS

The study was conducted with the participation of 35 Vietnamese military personnel volunteers (men) aged 19–32 years (average age 23.6 years), whose anthropometric data corresponded to the size and height of the high-altitude and protective equipment used.

All the participants had no acute illnesses or exacerbations of chronic states. Prior to each experiment, they underwent a medical examination in the scope of a preflight medical examination.

During the training period for the study, for each test subject, high-altitude and protective equipment was selected in accordance with their anthropometric parameters and the methodology for selecting and fitting high-altitude equipment [11]. The equipment was also fitted in accordance with this methodology. In this study, a KM-35 oxygen mask (KM-35) in combination with a ZSh-7A protective helmet (ZSh-7A) was used; a VKK-15 high-altitude compensating suit (VKK-15) was used to create counterpressure on the chest [15].

The direct creation of OOP was carried out using the BARS-GD hardware and software complex (BARS-GD, Russia). The registration of physiological and psychophysiological responses was carried out using the objective control system integrated into BARS-GD. This equipment includes a pneumatic system, a PC, an objective medical control system, and peripheral devices for conducting psychophysiological and stress tests (Fig. 1).

The developed course of breathing and speech training under OOP includes five OOP breathing sessions, which are conducted once a day for five consecutive days. The sessions were held in the morning, 1.5–2 h after a meal. On the day of the session, the test subjects were exempted from significant physical and psychological stress.

Each session consisted of breathing under OOP sequentially and continuously at five stages with the creation of overpressure at levels of 150–1000 mm H_2O; the breathing time at each stage was 2 min. BARS-GD was used in the mode of smooth increase of OOP in the airways. When conducting the session, VKK-15 was necessary due its mandatory use when creating an OOP of more than 300 mm H_2O in the human respiratory system.

To assess the impact of the five-day training course on the psychological and psychophysiological indicators of the test subjects, the breathing conditions of a rapid cockpit decompression at an altitude of 14–15 km were simulated using the BARS-GD in the mode of rapid creation of an OOP of 500 mm of water pressure in the respiratory tract.

Prior to and following OOP simulation, the psychological characteristics were assessed using the Spielberger–Khanin anxiety scale and the psychological state assessment method[2].

To assess the functioning of the central nervous system (CNS), the average time of a simple visual-motor reaction (SVMR) was evaluated, which characterizes the excitability and lability of the CNS, the mobility of nervous processes, and the balance of excitation and inhibition processes, by performing the tests of complex visual-motor reaction and response to a moving object (SVMR and RMO) [17]. These tests were performed by the test subjects during each training session, as well as during the simulation of rapid decompression.

The training sessions and rapid decompression simulations were performed in strict accordance with the

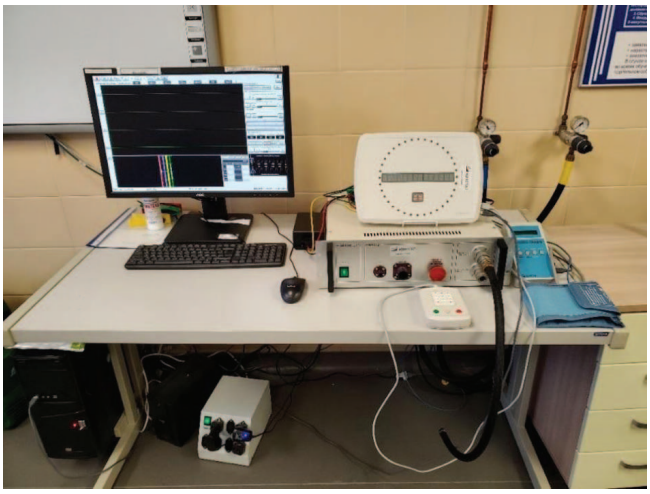

Photo taken by the authors

Fig. 1. Appearance of the BARS-GD equipment set

[2] Mantrova IN. Methodological guide to psychophysiological and psychological diagnostics. Ivanovo; 2005 (In Russ.).

indications, contraindications, and safety measures according to the approved methodology [11, 18].

The training effectiveness was evaluated in several ways. First, the participants' ability to perform respiratory movements under excessive pressure was assessed, since the biomechanics of this process involve significant physical effort to overcome the counterpressure during exhalation, leading to muscle fatigue in the chest and diaphragm within a few minutes. Secondly, the participants' ability to pronounce control words under these conditions was evaluated, since the ability to maintain radio communication is a key indicator of the professional reliability of pilots. Thirdly, the participants' psychophysiological reserves under the conditions of OOP were assessed based on the severity of physiological reactions, the level of situational anxiety, well-being, activity, and mood (WAM).

Mathematical and statistical analysis was carried out using the Microsoft® Excel-2016 software, SPSS 26 application package, with calculation of Student's t-test and Wilcoxon test. The values are presented in the form of median value and quartile spread. The dynamics of indicators without load and after load was compared separately in pre-training and post-training periods. The dynamics of indicators between the periods before and after the training course was also compared. All differences were considered statistically significant at a *p*-value of < 0.05.

RESULTS

The conducted assessment of personal anxiety found that 23 (66%) and 12 (34%) of the participants demonstrated a high and average level of anxiety, respectively. No participants showed a low level of anxiety.

Significant changes in situational anxiety (SA) were recorded during the examination of the participants before and after the training course. In the absence of OOP load, the SA level decreased by 4% from 43.3 ± 0.9 to 41.6 ± 1.0 points ($p = 0.039$) after the training course. No statistically significant changes were observed in these indicators before and after the training course. This may indicate that, over the five-day training course, the participants have developed the required level of psychological readiness for the subsequent exposure to an extreme factor (Fig. 2).

A study of the dynamics of SA levels during the simulation of a rapid decrease in pressure in an aircraft pressurized cabin showed a downward trend before and after the training course; however, the data were not statistically significant.

An analysis of the dynamics of the participants' psychological state using the WAM questionnaire before the training course showed a statistically significant decrease in the well-being indicator by 3.7% ($p = 0.003$) (Fig. 3) and the mood indicator by 5.7% ($p = 0.035$) (Fig. 4), indicating that the participants were not prepared for new biomechanical patterns of breathing in the OOP conditions, as well as the occurrence of a number of uncomfortable sensations when using high-altitude and protective equipment. No statistically significant changes were observed when comparing these indicators before and after the training course.

Following the five-day training course, the pilots' levels of well-being and mood were similar to their pre-training levels. These results reflect the pilots' subjective assessment of their psychological state, which was characterized by their readiness to withstand extreme conditions, their adaptation to the biomechanics of breathing in the OOP environment, and their use of high-altitude and protective equipment.

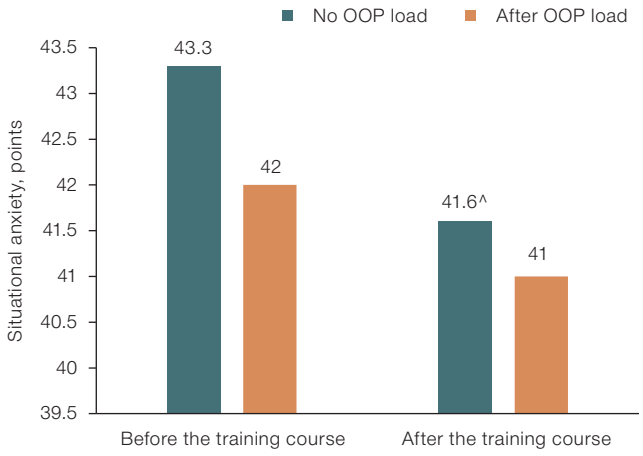

Figure prepared by the authors using their own data

Fig. 2. Situational anxiety changes during rapid creation of an oxygen overpressure (OOP) of 500 mmHg before and after exercise

Note: ^ — the level of statistical significance when comparing the unloaded indicator before and after the training course.

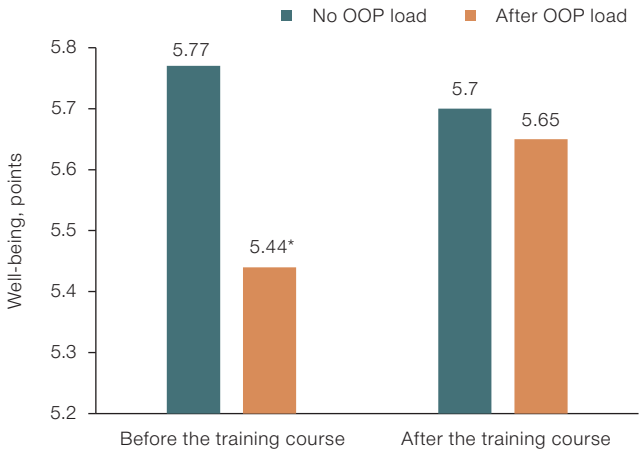

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Fig. 3. Well-being changes when oxygen overpressure (OOP) is rapidly increased to 500 mmHg before and after exercise

Note: * — the level of statistical significance ($p = 0.003$) when comparing the values before and after the training course.

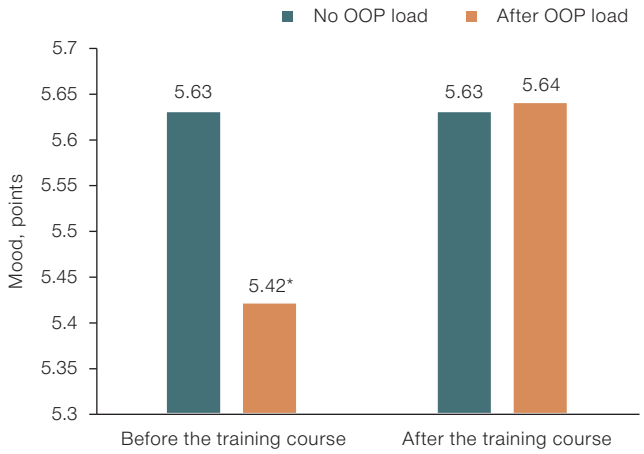

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Fig. 4. Changes in mood when oxygen overpressure (OOP) is rapidly increased to 500 mmHg before and after exercise

Note: * — the level of statistical significance (p = 0.035) when comparing the values before and after the training course.

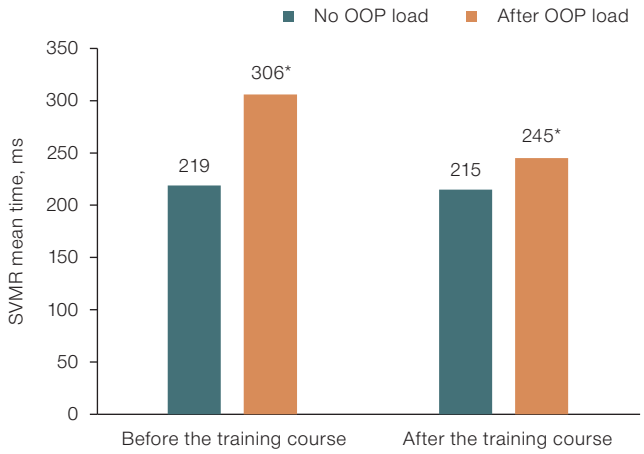

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Fig. 5. Dynamics of the mean time of simple visual-motor reaction (SVMR) during rapid creation of an oxygen overpressure (OOP) of 500 mmHg before and after training

Note: * — the level of statistical significance (p < 0.001) when comparing the values before and after exercise in the periods before and after the training course.

The study of the functional state of the CNS before the training course found that the average SVMR time of the participants increased significantly by 39.7% (p < 0.001) after the OOP load compared to the pre-load values; thus, after the training course, the SVMR time increased by 14.1% (p < 0.001). A comparison of the indicators before and after the training course did not show any significant changes. The obtained results reflect less significant changes in the level of CNS functioning after the training course than before the course, which is characterized by a less significant decrease in the speed of neural processes and, consequently, greater psychophysiological reserves (Fig. 5).

The dynamics of the mean CVMR time is presented in Fig. 6. Before the training course, the average CVMR time increased significantly, by 6.6% (p = 0.001), under the OOP load, while the number of erroneous responses remained at the same level. When comparing the CVMR time before and after the psychophysiological training course, the participants showed a decrease in the CVMR time (Fig. 6). At the same time, after the training course, there were no statistically significant changes in the time of the CVMR and the number of erroneous reactions compared to the pre-training values. These data indicate an increase in the psychophysiological reserves of the participants after the training course.

It is also noteworthy that there was a statistically significant decrease in the number of erroneous responses after the five-day training course, which was 65% (p < 0.001) in the absence of stress and 55% (p < 0.001) after exposure to OOP. These results indicate an increase in the CNS functioning under OOP conditions during training and an increase in the psychophysiological reserves of the participants.

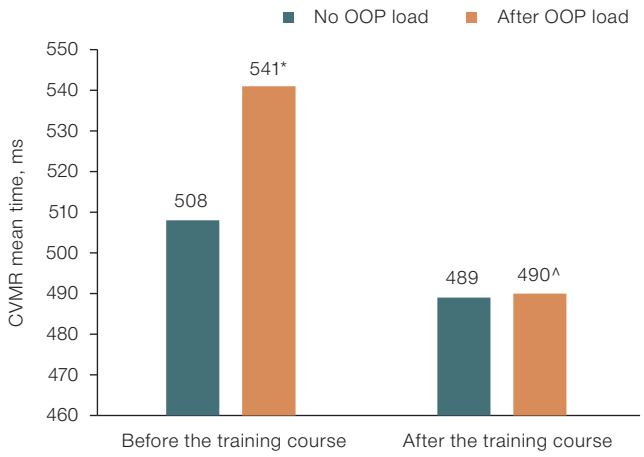

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Fig. 6. Dynamics of the mean time of complex visual-motor reaction (CVMR) during rapid creation of oxygen overpressure (OOP) at a level of 500 mm H_2O before and after training

Note: * — level of statistical significance (p = 0.001) when comparing indicators without and after exercise before the training course; ^ — level of statistical significance (p = 0.001) when comparing indicators after exercise before and after the training course.

The RMO time dynamics, which characterizes the balance of excitation and inhibition of CNS processes during the simulation of cockpit decompression, is presented in Fig. 7. Before the training course, the RMO time changed significantly, by 26.7% (p = 0.001), in the negative range of values from –58 to –73.5 ms. After the training course, the dynamics of this parameter did not have statistical significance, similar to the case when comparing the indicators before and after the training

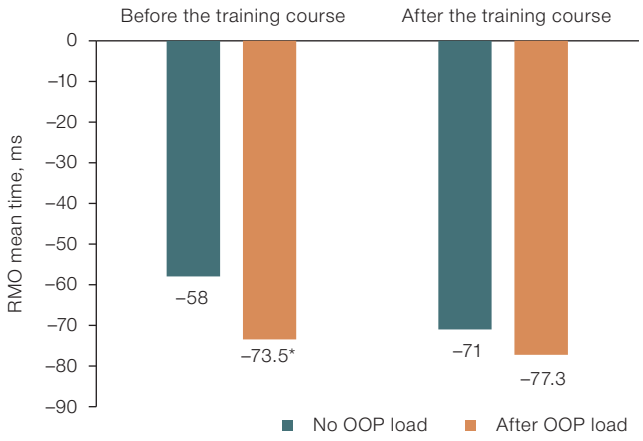

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Fig. 7. Mean time dynamics of response to a moving object (RMO) during rapid creation of an oxygen overpressure (OOP) of 500 mmHg before and after training

Note: * — the level of statistical significance (p = 0.001) when comparing the values before and after the training course.

course. From a physiological standpoint, these results are consistent with the changes in the SVMR and CVMR, characterizing the overall dynamics of a decrease in the CNS functioning level under the influence of an extreme flight factor, with the predominance of CNS excitation processes.

DISCUSSION

The conducted series of experiments have confirmed a significant effect of breathing under OOP on the psychological state and CNS functioning in test subjects who had no previous experience of such programs. However, after undergoing psychophysiological training and developing psychological and physiological readiness for exposure to OOP, statistically significant changes in psychophysiological indicators were recorded.

The five-day course of breathing and speech training under OOP among Vietnamese pilots improved their psychological readiness for exposure to extreme flight factors [2], which is evidenced by a statistically significant decrease in the participants' background situational anxiety and the absence of statistically significant changes in their well-being and mood after the training course. These are representative subjective indicators of psychological state. Given the non-physiological biomechanics of breathing under OOP conditions, which is characterized primarily by active exhalation, the pilot's psychological state plays a significant role in the success of their training [9]. In the context of stratospheric flights and sudden cockpit decompression, a pilot's lack of psychological readiness can pose a significant threat not only to the completion of the entire mission but also to the lives of the crew members and the safety of the aircraft [4].

Statistically significant changes in the psychophysiological indicators of the test subjects reflect the physiological aspect of their adaptation to breathing under OOP conditions and the use of high-altitude and protective equipment. The increase in psychophysiological reserves during the five-day training course indicates the formation of the corresponding breathing skills [13]. This mitigates the uncomfortable and limiting effects of high-altitude equipment, such as pressure on the face and neck, discomfort in the oral cavity, allowing pilots to conduct high-quality and clear radio communication.

CONCLUSION

The conducted research is part of a larger scientific project aimed at improving the safety and efficiency of piloting Russian-made jets in Southeast Asian countries. Modern aviation equipment places high demands on flight personnel during maneuvers and stratospheric missions. One of the challenges in medical support and training for Vietnamese flight personnel consists in the lack of an effective program for stratospheric flights, which can be conducted using Russian high-altitude and protective equipment.

This study showed the effectiveness of the developed five-day training course in maintaining the professional performance of Vietnamese test pilots under the conditions of a rapid depressurization of the aircraft cabin and breathing under OOP. The course effectiveness was confirmed, on the one hand, by the development of skills for performing an active exhalation with additional effort and for maintaining voice communication with the flight director and crew members, and, on the other hand, by statistically significant changes in psychological and psychophysiological indicators that demonstrate improved psychological readiness among test pilots and an increase in their psychophysiological reserves.

The conducted research provides grounds for recommending the breathing and speech training mode under OOP for inclusion in the medical support system for flights by Vietnamese pilots, particularly in terms of training for stratospheric and maneuverable flights on Russian-made jets.

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DEVELOPMENTAL FEATURES OF IMMERSION PULMONARY EDEMA IN DIVERS

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Introduction. Immersion pulmonary edema (IPE) is a pathological condition that occurs in an aquatic environment during various activities, such as underwater engineering, scuba diving, triathlon competitions, etc. Despite a significant number of English-language publications, the problem of IPE remains insufficiently studied in Russia.
Objective. Research into the diagnosis, clinical manifestations, treatment, and prevention of IPE to optimize medical care for this pathological condition.
Discussion. The main factors leading to IPE include exposure to cold water, intense physical exertion during swimming, increased blood pressure while in water, excessive fluid intake before swimming, age over 50. Breathing 100% oxygen underwater can cause hyperoxia, oxidative stress, disruption of the alveolar–capillary membrane integrity, and surfactant deficiency, leading to fluid transudation into the pulmonary interstitial tissue and edema. Hyperoxia induces pulmonary vasoconstriction, increases hydrostatic pressure, and enhances fluid filtration into the interstitium, exacerbating IPE and contributing to the development of alveolar pulmonary edema. Clinically, IPE presents with labored breathing, acute dyspnea, coughing with hemoptysis, frothy bloody discharge, and other symptoms. A distinctive feature of this condition is the resolution of key symptoms within 48 h. On physical examination, percussion over the affected lung area reveals dullness, while auscultation detects wet rales in the lungs and murmurs characteristic of acute mitral regurgitation with left ventricular failure. Computed tomography findings include ground-glass opacities, peribronchial infiltration, and pleural effusion, predominantly on the affected side. A major limitation of this method is the inability to perform imaging immediately during an emergency ascent. Ultrasound diagnostic markers of IPE include hyperechoic reverberation artifacts (B-lines), produced by the interaction of ultrasound waves with air-fluid content in the alveoli, typical of pulmonary edema. Clinical and laboratory markers of IPE include elevated levels of copeptin, brain natriuretic peptide (BNP), ischemia-modified albumin, and high-sensitivity troponin T.
Conclusions. IPE remains an understudied yet highly dangerous pathological condition in diving and aquatic swimming. Therefore, it is crucial to educate divers, combat swimmers, professional scuba divers, and athletes (triathletes, swimmers) about preventive measures and symptom recognition when they occur during surface or underwater activities. Implementing a comprehensive approach to IPE prevention will reduce the incidence of this condition and enhance the safety of diving operations.

Keywords: immersion pulmonary edema; IPE; diving; scuba diving; risk factors; pathological condition; diving operations; triathlon; drowning; pulmonary barotrauma; thoracic compression; gas embolism; pulmonary edema symptoms

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ОСОБЕННОСТИ РАЗВИТИЯ ИММЕРСИОННОГО ОТЕКА ЛЕГКИХ У ВОДОЛАЗОВ

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Введение. Иммерсионный отек легких (ИОЛ) — патологическое состояние, которое возникает в водной среде при различных видах деятельности: подводно-технических работах, любительском дайвинге, спортивных соревнованиях по триатлону и т.д. Несмотря на значительное количество англоязычных публикаций, в России проблема ИОЛ остается недостаточно изученной.
Цель. Исследование особенностей диагностики, клинических проявлений, лечения и профилактики иммерсионного отека легких для оптимизации медицинской помощи при данном патологическом состоянии.
Обсуждение. К основным факторам, приводящим к ИОЛ, относятся: нахождение в холодной воде, тяжелая физическая нагрузка при плавании, повышенное артериальное давление в период нахождения в воде, избыточное потребление жидкости перед плаванием, возраст свыше 50 лет; дыхание под водой 100% кислородом, вызывающим гипероксию, оксидативный стресс, нарушение целостности альвеоло-капиллярной мембраны и дефицит сурфактанта, что приводит к транссудации жидкости в интерстициальную ткань легких и отеку. Воздействие гипероксии приводит к вазоконстрикции легочных сосудов, повышению гидростатического давления и усилению фильтрации жидкости в интерстиции, что усугубляет развитие ИОЛ и обусловливает развитие альвеолярного отека легких. Клинически ИОЛ проявляется затрудненным дыханием, острой одышкой, кашлем с кровохарканьем, кровянистыми выделениями пенистой консистенции и другими симптомами. Отличительной чертой паталогического состояния является исчезновение основных симптомов в течение 48 часов. Вместе с тем при проведении перкуссии над пораженным участком легкого отмечается притупление звука, при аускультации в легких выслушиваются влажные хрипы; при аускультации сердца — шумы, характерные для острой митральной недостаточности с левожелудочковой недостаточностью. При компьютерной томографии выяв-

ляются снижение прозрачности легочных полей по типу «матового стекла», перибронхиальная инфильтрация и плевральный выпот, преимущественно на пораженной стороне. Основным ограничением метода является невозможность проведения исследования непосредственно в условиях аварийного спуска. Ультразвуковыми признаками диагностики ИОЛ можно считать наличие гиперэхогенных реверберационных артефактов (B-линий), образующихся при взаимодействии ультразвуковых волн с воздух-жидкостным содержимым альвеол, характерным для отека легких. Клинико-лабораторным маркером ИОЛ является повышение копептина, мозгового натрийуретического пептида, модифицированного ишемией альбумина, сверхчувствительного тропонина Т.
Выводы. ИОЛ остается недостаточно изученным, но крайне опасным патологическим состоянием в дайвинге и плавании на воде, поэтому водолазам, боевым пловцам, дайверам и спортсменам (триатлонистам, пловцам) целесообразно доводить информацию о мерах предосторожности при появлении его симптомов во время плавания на воде и под водой. Применение комплексного подхода в профилактике ИОЛ снизит частоту случаев появления патологического состояния и повысит безопасность водолазных спусков.

Ключевые слова: иммерсионный отек легких; ИОЛ; дайвинг; подводное плавание; факторы риска; патологическое состояние; водолазные спуски; триатлон; утопление; баротравма легких; обжатие грудной клетки; газовая эмболия; симптомы отека легких

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INTRODUCTION

The occupational activities of divers predispose them to specific pathological conditions including decompression sickness, ear and pulmonary barotrauma, thoracic compression, arterial gas embolism, and nitrogen narcosis (a pathological state induced by the toxic effects of nitrogen when breathing air at depths exceeding 30 m). International medical practice also recognizes immersion pulmonary edema (IPE) as a potentially fatal emergency condition during submersion [1, 2]. IPE is an acute pathological condition occurring during surface or underwater swimming, frequently affecting individuals without previous history of cardiovascular pathology (e.g., ischemic heart disease, acute/chronic heart failure, or cardiomyopathies) [3].

The actual prevalence of IPE is unknown; however, there are reports of an estimated range of 1.8% (in experienced) to 60.0% (in novice) among combat swimmers, and 1.4% among triathletes. IPE can cause severe clinical manifestations, including acute respiratory distress, hemoptysis due to erythrocyte diapedesis, paroxysmal supraventricular tachycardia, and syncope caused by myocardial hypoxia and cerebral hypoperfusion [3, 4]. At the same time, minor manifestations in divers are rarely described in the literature [3, 5]. Most of the publications on the IPE problem are presented in the form of clinical cases and focused retrospective studies. In the Russian-language literature, separate references to the manifestations of IPE can be found, mainly describing atypical forms of already known diseases (drowning, chest compression, pulmonary barotrauma, arterial gas embolism, etc.).

Fatal cases of IPE are rarely documented due to the diagnostic challenge of distinguishing them from other diving-related fatalities (drowning, ischemic heart disease, pulmonary barotrauma, etc.) [6, 7]. To date, no autopsy-confirmed cases of IPE have been reported in Russia, whereas there exist internationally established diagnostic criteria to differentiate this condition from other pathologies [8–10].

In this article, we aim to analyze the diagnostic markers, clinical presentation, management protocols, and preventive strategies for IPE to optimize the respective therapeutic interventions.

MATERIALS AND METHODS

We conducted a systematic literature review incorporating prospective and retrospective studies along with clinical case analyses. Our search strategy included international (PubMed, MEDLINE, Embase, Cochrane Library, Scopus, Web of Science) and Russian (eLibrary, CyberLeninka, RSCI) databases using the following keywords individually and in combination: immersion, exercise-induced, cold-induced, pulmonary edema, hemodynamics, cardiovascular response, water immersion, drowning, thoracic compression, pulmonary barotrauma, pulmonary edema, pulmonary hypertension, and cold shock response.

RESULTS AND DISCUSSION

Clinical epidemiology

The first documented cases of immersion pulmonary edema (IPE) were reported by Wilmshurst et al. in 1981, occurring in 71 individuals (0.18%) participating in open-water swimming events [8]. All affected individuals were ostensibly healthy adolescents aged 18–19 years. At that time, IPE diagnosis was confirmed based on the acute onset of severe dyspnea and coughing during

or immediately after swimming, coupled with auscultatory findings of pulmonary edema (inspiratory crackles throughout all lung fields) [11].

Pons et al. (1995) conducted a survey of 460 divers, identifying only 5 cases (1.1%) with IPE symptoms, including exercise-induced respiratory distress, involuntary coughing, hemoptysis (with or without frothy sputum production). With the purpose of investigating IPE pathophysiology, the researchers evaluated forearm vascular resistance, vasoactive hormone levels, biventricular function (via Doppler echocardiography) under both normothermic and cold stress conditions. Characteristic IPE hemodynamic changes were observed in just 1 out of 10 study participants [12].

A 2002 study by Mahon et al. revealed frequent IPE occurrences among U.S. Navy SEAL candidates undergoing intensive training, with an annual incidence of about 20 cases, predominance among recruits, documented recurrence in some operators, suggesting individual predisposition [13].

A review 1400 U.S. triathletes conducted by Miller et al. identified 20 cases (1.4%) with definitive IPE symptoms (exercise-associated hemoptysis with frothy secretions). The study established systemic hypertension and left ventricular hypertrophy to be the key risk factors. These conditions promote diastolic dysfunction, increasing myocardial preload and afterload [14]. Current epidemiological data indicate IPE incidence rates of 1.1% in professional divers and 1.8% among experienced combat swimmers, technical divers, triathletes [4, 11, 15–17].

Risk factors and pathogenesis

The main factors leading to immersion pulmonary edema (IPE) include exposure to cold water [13, 18, 19–21], intense physical exertion while swimming [22], elevated blood pressure during immersion [11], excessive fluid intake before swimming [13], and age over 50 [22]. Another common cause of IPE is breathing 100% oxygen underwater [19], which induces hyperoxia, oxidative stress, disruption of the alveolar-capillary membrane integrity, and surfactant deficiency, leading to fluid transudation into the pulmonary interstitium and edema. Hyperoxia causes pulmonary vasoconstriction, increased hydrostatic pressure, and enhanced fluid filtration into the interstitium, exacerbating IPE and causing alveolar pulmonary edema [23].

Consumption of 1–3 liters of water before swimming was found to increase the risk of IPE [18, 23]; however, cases where IPE occurred without prior fluid loading were also reported. An increase in circulating blood volume contributes to IPE due to resulting hypertension in the pulmonary circulation [20, 21]. The study [22] demonstrated a correlation between aspirin intake or fish oil consumption and IPE.

Three groups of factors contribute to the development of IPE:

1. *Physiological factors* (arterial hypertension, left ventricular hypertrophy, cardiac arrhythmia). Physically fit individuals and athletes may be more susceptible to IPE [10, 24], largely due to the high prevalence of left and right ventricular hypertrophy [21] and reduced cardiac chamber compliance caused by physical training [6]. Individuals with functional cardiovascular changes, such as left ventricular hypertrophy, are at a higher risk of IPE [8, 16]. Reduced lung volume due to compression under increased pressure can trigger IPE. A low number of interlobular septa and lymphatic vessels in the lungs also contributes to IPE predisposition by impairing the clearance of alveolar fluid via the lymphatic system [9, 21, 23].

2. *External factors* (cold water, intense physical exertion, depth and duration of immersion). Low water temperatures and ill-fitting wetsuits stimulate peripheral vasoconstriction, increasing preload on the left and right ventricles, thus elevating pressure in the left heart chambers and pulmonary artery [10, 20]. Exposure to cold temperatures leads to blood flow centralization, further increasing pulmonary vascular pressure, while a tight wetsuit worsens blood flow in these vessels [25].

3. *Individual characteristics* (female sex, age, obesity) [5, 6, 22]. Females are more prone to IPE, possibly due to anatomical and physiological differences in the cardiovascular system and hormonal influences. Older individuals face a higher risk of IPE due to age-related changes in the heart, blood vessels, and lungs. Excess weight places additional strain on the cardiovascular and respiratory systems, particularly during physical activity in water.

The pathogenesis of IPE is primarily driven by physiological and pathophysiological processes leading to pulmonary edema.

Hydrostatic pressure and blood flow centralization [26]. Immersion in water increases external pressure (especially in an upright position), compressing peripheral vessels and enhancing venous return to the heart. This raises pressure in the right atrium and pulmonary vessels [20].

Increased pulmonary capillary pressure (capillary stress). Elevated circulating blood volume increases hydrostatic pressure in pulmonary capillaries. When it exceeds 25–30 mmHg, transudation of fluid into the pulmonary interstitium intensifies [27].

Cold stress and vasoconstriction. Cold water triggers reflexive peripheral vasoconstriction to preserve core temperature, redistributing blood to central organs and further increasing pulmonary vascular load [28].

Impaired cardiac function. Some divers, particularly those with long-term experience, exhibit reduced myocardial adaptability to hemodynamic stress during dives. This leads to elevated pulmonary capillary pressure and acute cardiogenic pulmonary edema [23, 29].

Endothelial damage and inflammation. Hypoxia, mechanical vascular stretching, and oxidative stress injure

capillary endothelium. Inflammatory mediators (histamine, bradykinin) are released, increasing vascular permeability [30].

Lymphatic drainage insufficiency [31]. The lymphatic system fails to clear excess fluid from the lungs, exacerbating edema progression.

Independent studies by Kumar et al. indicate that a prone (face-down) position during immersion intensifies blood flow centralization, contributing to unilateral IPE linked to lateral positioning [3, 22]. The design features of breathing apparatuses can alter inspiratory/expiratory airflow, further promoting IPE.

In rebreather diving, the regulation via an automated gas supply valve creates high inspiratory resistance and restricted ventilation. This underscores the critical role of negative-pressure breathing in interstitial pulmonary edema development. Such breathing reduces intrathoracic, airway, and interstitial pressures, elevating capillary pressure with each breath. Repeated dives and ascents cause interstitial fluid accumulation, triggering pathological decompensation and IPE symptoms [4, 24].

Moreover, over 20% of hospitalized divers with IPE report prior episodes with high recurrence rates, supporting the concept of individual susceptibility. Growing evidence suggests genetic predisposition linked to polymorphisms in genes encoding surfactant proteins and endothelial growth factors [32].

Clinical Presentation and Diagnosis of Immersion Pulmonary Edema

Clinical symptoms of IPE in the presence of triggering factors (cold water, strenuous exertion, breathing 100% oxygen, etc.) may appear within 10–30 min of being in the aquatic environment [6]. In 90% of divers with IPE, dyspnea, cough, and sputum production are observed [11, 12, 19, 46], while approximately 50% experience hemoptysis [11, 18].

According to Adir et al., other symptoms (weakness, orthopnea, chest discomfort [11], dizziness [11, 12, 9], and loss of consciousness [24]) occur less frequently. Percussion over the affected area of the lung reveals dullness, which may indicate infiltration, pleural effusion, or other pathological processes [11]. On auscultation, wet rales are heard in the lungs, while cardiac auscultation may reveal murmurs characteristic of acute mitral insufficiency with left ventricular failure (gallop rhythm (S3) and pansystolic murmur of mitral regurgitation) [12]. Pulse oximetry typically shows tissue oxygen saturation below 85% [11, 8].

The differential diagnosis of IPE is generally performed with cardiovascular diseases, drowning, thoracic compression, respiratory failure, pulmonary barotrauma, and bronchial asthma [24, 33–35].

Chest X-ray examinations conducted within the first 12–18 h after IPE onset may reveal no pathological changes [11, 22]. However, typical radiographic signs may later appear, including pulmonary artery dilation, redistribution of blood flow to the upper lung lobes (cephalization), and Kerley B lines, reflecting interstitial or alveolar edema with thickening of interlobular septa [12, 13, 17, 37]. Computed tomography reveals ground-glass opacities [36], peribronchial infiltration, and pleural effusion [37, 38], predominantly on the affected side, which is associated with increased blood flow, pressure gradient, and the development of mitral regurgitation [13, 22, 39].

During lung ultrasound, parenchymal visualization is possible in the presence of pathological changes accompanied by reduced alveolar aeration, allowing the ultrasound beam to partially penetrate the interlobular septa. Diagnostic ultrasound signs of IPE include hyperechoic reverberation artifacts (B-lines), which form due to the interaction of ultrasound waves with the air-fluid content of alveoli, characteristic of pulmonary edema. Concurrently, A-lines are observed as horizontal hyperechoic structures resulting from reflection artifacts off the visceral pleura, spaced at equal intervals from one another and from the pleural line. The barcode sign is a diagnostically significant finding, indicating the cessation of lung sliding and the presence of pneumothorax as a consequence of barotrauma. Lung ultrasound allows differentiation between immersion pulmonary edema and barotrauma-induced injury, while quantitative assessment of B-lines enables determination of the degree of interstitial or alveolar edema. This method is of high diagnostic value due to its simplicity, non-invasiveness, and rapid execution [35, 38, 40].

Computed tomography is the most informative and sensitive diagnostic method for IPE [36, 41]. Characteristic signs of IPE include pleural effusion, ground-glass opacities with lobar distribution, and thickening of interlobular septa [41]. The main limitation of this method is the inability to perform the examination directly during an emergency dive.

Zavorsky et al. and Gempp et al. have identified electrocardiographic changes in IPE, manifested as nonspecific myocardial repolarization disturbances (ST-segment elevation/depression), reflecting hypoxic myocardial injury [38, 42].

Clinical and laboratory markers of IPE include elevated levels of:

- copeptin (an indirect indicator of antidiuretic hormone activity);
- brain natriuretic peptide (BNP);
- ischemia-modified albumin;
- high-sensitivity troponin T.

It was shown in [32, 43, 44] that the combination of elevated troponin T and BNP has the highest diagnostic specificity for differentiating IPE, confirming the cardiogenic component of its pathogenesis. According to the same researchers, echocardiography in IPE reveals signs of systolic dysfunction, such as global or regional

hypokinesia, reduced ejection fraction, left ventricular hypertrophy [31, 38, 42, 44].

Patients with IPE exhibit restrictive ventilation patterns, including decreased forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) with preserved Tiffeneau index (FEV1/FVC) [11, 16, 38].

Additionally, reductions were observed in absolute FVC values, decreased maximum expiratory flow rates at 25% and 75% of forced vital capacity (MEF25%, MEF75%), and impaired lung diffusion capacity [11]. While lung diffusion capacity normalizes within 24 h, other pulmonary function parameters typically recover within approximately one week.

According to Casey et al., bronchoalveolar lavage analysis revealed the presence of erythrocytes and high-molecular-weight proteins (albumins and globulins) in the lavage fluid. Notably, systemic inflammation markers (C-reactive protein, procalcitonin, neopterin, presepsin, tumor necrosis factor-α), complete blood count parameters (leukocytes, neutrophils, ESR), and protein profile indicators (total protein, IgM) remained within normal reference ranges [45].

Treatment and prevention

The IPE treatment is based on clinical guidelines for managing pulmonary edema patients, due to the lack of randomized controlled trials in divers [35, 46–48]. Primary interventions include immediate extraction from water, transfer to a warm environment, and removal of the wetsuit/dry suit. Secondary measures involve oxygen therapy and pharmacological support (diuretics, β_2-agonists, and antibiotics and corticosteroids when indicated).

With prompt treatment, 82% of patients experience complete resolution of symptoms within 48 h [30, 47–50]. However, recurrence rates in certain groups (particularly athletes) range from 13–75% [20, 49, 50]. According to Shupak et al., 75% of cases demonstrate progressive clinical manifestations during recurrent episodes, indicating individual predisposition [20, 49, 50].

Preventive measures include administration of dihydropyridine calcium channel blockers and selective PDE5 inhibitors (sildenafil) prior to diving to reduce systemic blood pressure and pulmonary hypertension [35, 46]. Mechanism of action is vasodilation (reducing smooth muscle tone and increasing venous capacitance), which may minimize IPE risk.

Clinical cases of IPE development and their analysis

We analyzed 80 incident reports and identified 16 cases that best matched the clinical presentation of IPE. In this article, we report four representative cases of IPE in divers. Among them, the first case report details the author's personal experience in diagnosing and managing IPE in a professional diver [7].

Case 1. The case involved a 35-year-old male diving instructor (designated as Z.) with one year of diving experience and 25 logged diving hours. During a dive in the Black Sea using an IDA-71P apparatus at 6 m depth, the diver experienced respiratory distress and involuntary coughing that forced him to abort the dive. Upon surfacing and boarding the support vessel, he continued coughing, producing bloody, frothy, bright red sputum. Initial physical examination revealed no pathological findings on auscultation or percussion, although chest radiography showed increased pulmonary vascular markings in the lower lung fields.

To confirm the diagnosis, a thoracic computed tomography (CT) scan was performed, revealing evidence of fluid accumulation in the lung tissue. Based on these findings, the preliminary diagnosis was pulmonary barotrauma, and therapeutic recompression was initiated using Protocol II. The diver reported symptomatic improvement during compression at 0.8 MPa. Follow-up CT imaging after completion of recompression therapy showed complete resolution of the previously observed pathological changes. Following medical rehabilitation, the diver successfully returned to professional diving activities [7].

The chest CT scan (axial projection, the lung window at the level of the right ventricular outflow tract) of diver Z. demonstrated marked irregular pulmonary aeration patterns resulting from hemorrhagic infiltration with thickening of interacinar and interlobular septa — presenting as a ground-glass opacity pattern (Fig. 1A). Additionally, thickening of interacinar and interlobular septa was observed with alveolar spaces filled by hyperdense material (blood), predominantly located in subpleural regions of the posterior and lateral segments S_{III}–S_{IX} of the right lung and S_{III}–S_{V} of the left lung (Fig. 1B). The follow-up CT scan performed after therapeutic recompression showed complete resolution of all previously identified pathological findings (Figs. 1C and 1D).

It should be noted that diver Z's radiographic findings were not characteristic of typical clinical forms of pulmonary barotrauma. The tomogram revealed signs of pronounced pulmonary edema without evidence of emphysema or pneumothorax, which are hallmark features of pulmonary barotrauma. At that time, the evident pulmonary edema was classified as an atypical form of pulmonary barotrauma. Moreover, the pathogenesis and clinical manifestations of this pathological condition distinguished it from the typical form of pulmonary barotrauma, which results from pulmonary hypertension.

Consequently, in this case, the most probable cause of the emergency situation was the development of IPE, as the clinical symptoms were specifically characteristic of this pathology.

Case 2. Diver A. was performing a dive using an IDA-71P closed-circuit rebreather to a depth of 7 m. After 25 min underwater, diver A. surfaced and reported severe coughing and chest pain. Light brown inclusions were observed in the diver's saliva. The diving physician diagnosed pulmonary barotrauma and performed therapeutic recompression, resulting in a successful outcome [7].

Case 3. Diver C. descended to 10 m using a closed-circuit breathing apparatus. After 40 min, the diver stopped responding to status checks and was brought to the surface unconscious by the safety diver. Upon regaining consciousness, diver C. complained of retrosternal pain (worsening with inspiration), progressive weakness, and coughing. Pink, frothy sputum was observed. The diving physician diagnosed pulmonary barotrauma and conducted therapeutic recompression with full recovery [38]. No differential diagnosis was performed, the root cause of the incident remained undetermined.

Thus, in the above three cases, the divers were diagnosed with pulmonary barotrauma and underwent therapeutic recompression, as there was insufficient evidence to confirm IPE (no chest CT or lung ultrasound data available). This decision was made to prevent arterial gas embolism. A potential solution in such situations would be to perform differential diagnosis using lung ultrasound to detect A- or B-lines, which serve as diagnostic criteria for pulmonary barotrauma and IPE.

Case 4. Recreational divers K. and M. conducted a dive using an AVM-5 apparatus to a depth of 7 m for amber collection in water at +3°C and air temperature of –7°C. No other individuals were present at the dive site. Upon surfacing, diver M. noticed that diver K. was missing. The following day, rescue personnel recovered diver K.'s body. Inspection of the equipment confirmed the AVM-5 was functional, with the cylinder pressure of 20 MPa and air quality meeting regulatory standards. The investigative commission concluded that the fatality resulted from drowning, hypothesizing that the AVM-5 regulator of diver K. froze in the extreme cold, ceasing air delivery. Experiencing breathing difficulties underwater, diver K. expelled the mouthpiece but failed to surface due to negative buoyancy [7].

Research on cold-water immersion demonstrates that hemodynamic changes induced by cold exposure elevate pulmonary artery pressure and increase pulmonary ventilation, disrupting the alveolar–capillary barrier.

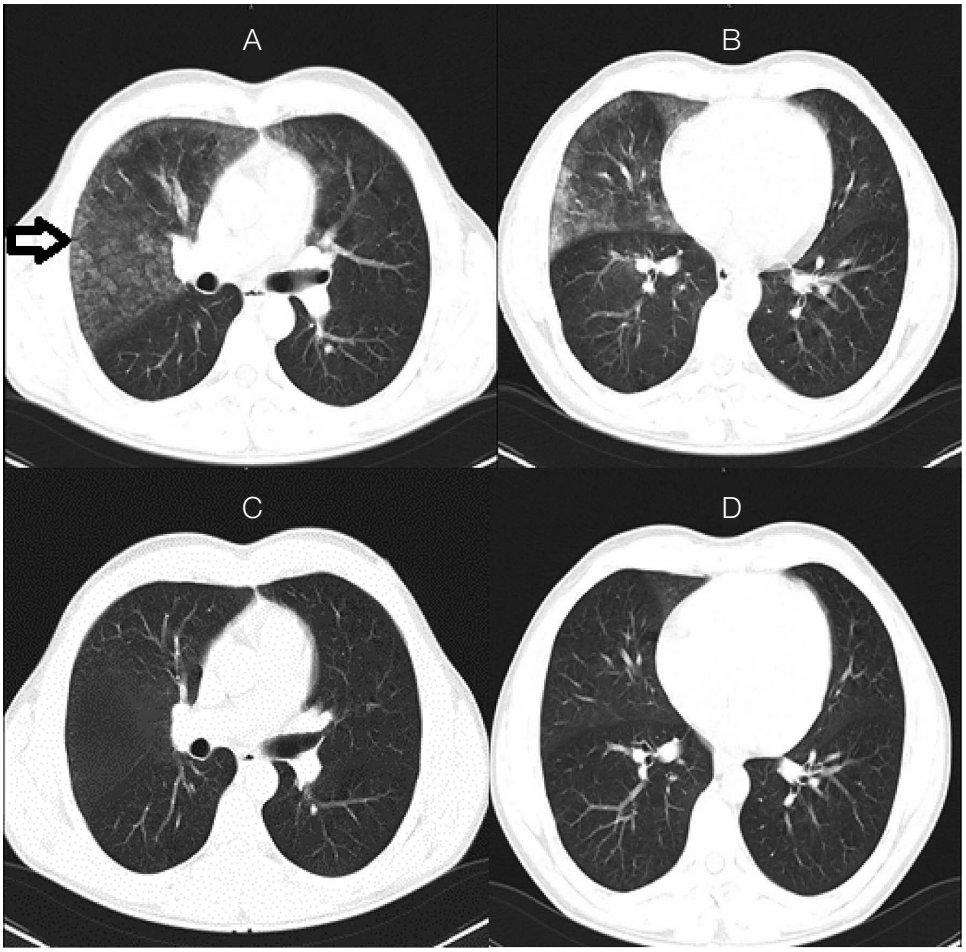

Figure prepared by the authors based on their own data

Fig. 1. Computed tomography of the thorax in diver Z. demonstrating ground-glass opacity (arrow)

These alterations promote fluid transudation into alveolar spaces.

This case bears strong resemblance to IPE-related incidents described in international literature [8, 11, 13, 18], where cold water exposure constitutes the primary risk factor for this pathology. This factor, particularly when combined with strenuous underwater exertion, frequently leads to IPE and subsequent drowning.

CONCLUSION

Despite the considerable number of English-language publications on immersion pulmonary edema (IPE), this subject remains insufficiently studied in Russia. The epidemiology of IPE varies significantly across different populations: the highest incidence (up to 60%) is observed among combat swimmer recruits, while among triathletes, swimmers, and professional divers it does not exceed 1.8%.

The key risk factors for IPD include hypothermia, hypertension, physical exertion in aquatic environments, overhydration, breathing hyperoxic gas mixtures, and age over 50 years. The primary IPE symptoms are inspiratory dyspnea and non-productive cough progressing to bloody sputum production.

The gold standard diagnostic methods for IPE are chest CT scans and lung ultrasound examinations. Due to the lack of evidence-based protocols, treatment remains symptomatic and follows general clinical guidelines for managing pulmonary edema. The main preventive measure involves medical screening to identify individuals predisposed to IPE development.

The conducted analysis of diving accidents in Russia confirmed the occurrence of cases with IPE symptomatology. Promising research directions include in-depth investigation of IPE pathogenesis and development of rapid diagnostic methods that could enable immediate identification of this condition at dive sites.

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BONE METABOLISM MARKERS IN YOUNG HIGH-PERFORMANCE ATHLETES

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Introduction. When assessing bone metabolism markers in athletes under the age of 18, it should be borne in mind that, in comparison with adults, the pediatric population is characterized by higher values of these markers. Their maximum increase during puberty coincides with peak bone mass gain.
Objective. To evaluate bone metabolism status in healthy high-performance athletes under the age of 18 based on the levels of C-terminal telopeptide (β-CrossLaps), osteocalcin, and N-terminal propeptide human procollagen type 1 (P1NP) in the blood serum.
Materials and methods. A single-center, cross-sectional study involved 383 juvenile athletes aged 13–18 years (248 girls and 135 boys; average age 15.2 [14.0; 16.1] years) from Russian national sports teams. The study was conducted in the period from March 2021 to July 2023. All athletes were divided into groups according to age and gender. The male groups were as follows: 13.1–14.0 years old (n = 3); 14.1–15.0 years old (n = 11); 15.1–16.0 years old (n = 43); 16.1–17.0 years old (n = 42); and 17.1–18.0 years old (n = 36). The female groups were as follows: 13.1–14.0 years old (n = 17); 14.1–15.0 years old (n = 51); 15.1–16.0 years old (n = 65); 16.1–17.0 years old (n = 59); and 17.1–18.0 years old (n = 56). The serum levels of osteocalcin, C-terminal telopeptide, and procollagen type 1 were evaluated in all athletes. The sexual maturity rating (SMR) was assessed according to the Tanner Scale. Statistical data processing was performed using the Statistica 10.0 software package (StatSoft Inc.; USA).
Results. The maximum values of β-CrossLaps in boys (2.27 [1.14; 3.45] ng/mL) and girls (1.55 [1.10; 2.02] ng/mL) were observed at the age of 13–14 years. The levels of osteocalcin and P1NP in young high-performance athletes corresponded to the standards for children with a normal level of physical activity. The maximum values of P1NP were revealed at the age of 13–14 years in both male (767.8 [148.1; 1142.4] ng/mL) and female (450.5 [268.6; 569.3] ng/mL) groups. In boys, the maximum values of osteocalcin (125 [89; 144] ng/mL) were detected at the age of 14–15 years; in girls (86 [62; 131] ng/mL) — at the age of 13–14 years.
Conclusions. In young high-performance athletes, the β-CrossLaps level as the main marker of bone resorption significantly exceeds the population norms for children and adolescents with a normal level of physical activity. When assessing the level of β-CrossLaps, osteocalcin, and P1NP, reference values should be adjusted to account for the gender and sexual maturity stage of athletes. The data obtained can be used when interpreting the results of an in-depth medical examination of athletes from Russian national sports teams to identify bone remodeling disorders.

Keywords: children; young athletes; sports medicine; osteocalcin; β-CrossLaps; P1NP

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МАРКЕРЫ МЕТАБОЛИЗМА КОСТНОЙ ТКАНИ У ЮНЫХ ВЫСОКОКВАЛИФИЦИРОВАННЫХ СПОРТСМЕНОВ

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Введение. При оценке маркеров костного метаболизма у спортсменов, не достигших 18-летнего возраста, следует учитывать, что для педиатрической популяции характерны более высокие значения данных метаболитов по сравнению со взрослой, а их максимальное повышение в период пубертата совпадает с пиковым набором костной массы.
Цель. Оценить состояние метаболизма костной ткани по уровням С-концевого телопептида (β-CrossLaps), остеокальцина и N-терминального пропептида человеческого проколлагена 1-го типа (P1NP) в сыворотке крови у здоровых высококвалифицированных спортсменов, не достигших 18-летнего возраста.
Материалы и методы. Проведено одномоментное одноцентровое исследование, в котором участвовали 383 юных спортсмена в возрасте 13–18 лет (из них 248 девочек и 135 мальчиков; средний возраст 15,2 [14,0; 16,1] года) сборных команд Российской Федерации в период с марта 2021 по июль 2023 г. Все спортсмены были разделены на половозрастные группы: мальчики: 13,1–14,0 (n = 3); 14,1–15,0 (n = 11); 15,1–16,0 (n = 43); 16,1–17,0 (n = 42); 17,1–18,0 года (n = 36); девочки: 13,1–14,0 (n = 17); 14,1–15,0 (n = 51); 15,1–16,0 (n = 65); 16,1–17,0 (n = 59); 17,1–18,0 года (n = 56). У спортсменов определяли уровень остеокальцина, С-концевого телопептида, проколлагена 1-го типа в сыворотке крови. Оценка полового развития проведена по классификации Tanner. Статистическая обработка данных произведена с использованием пакета прикладных программ Statistica version 10.0 (StatSoft Inc., США).
Результаты. Установлено, что максимальные значения β-CrossLaps у мальчиков (2,27 [1,14; 3,45] нг/мл) и девочек (1,55 [1,10; 2,02] нг/мл) отмечены в возрасте 13–14 лет. Уровни остеокальцина и P1NP у юных высококвалифицированных спортсменов соответствовали нормам для детей с обычным уровнем физической активности. Максимальные значения P1NP определялись в возрасте 13–14 лет как у мальчиков (767,8 [148,1; 1142,4] нг/мл), так и у девочек (450,5 [268,6; 569,3] нг/мл). Максимальные значения остеокальцина у мальчиков (125 [89; 144] нг/мл) достигаются в возрасте 14–15 лет; у девочек (86 [62; 131] нг/мл) — в возрасте 13–14 лет.
Выводы. Уровень β-Cross laps — основного маркера костной резорбции — у юных высококвалифицированных спортсменов значительно повышен по сравнению с популяционными нормами для детей и подростков с обычным уровнем физической активности. При оценке уровня β-CrossLaps, остеокальцина и P1NP целесообразно применение референтных значений с учетом пола и стадии полового развития спортсменов. Полученные данные могут быть использованы при интерпретации результатов углубленного медицинского обследования у спортсменов спортивных сборных команд РФ для выявления нарушений ремоделирования костной ткани.

Ключевые слова: дети; юные спортсмены; спортивная медицина; остеокальцин; β-CrossLaps; P1NP

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INTRODUCTION

Assay of bone metabolism markers is an effective diagnostic tool for assessing the functional status of the skeletal system in clinical practice [1–4]. However, high growth rates in children (especially in adolescents) intensify the processes of bone metabolism and, compared to adults, are associated with higher values of bone metabolism markers. Intense and prolonged physical activity performed by high-performance athletes can also affect the level of these markers [5, 6]. The syndrome of relative energy deficiency in sport (RED-S) is associated with a decreased acquisition of bone mass and bone microarchitectonics disorders in adolescence [2–4]. The hypothalamic amenorrhea (for girls) and functional hypogonadotropic hypogonadism (for boys), as part of RED-S, combined with vitamin D deficiency, is an additional risk factor for fractures in professional athletes, especially under the age of 18 [2, 5–8].

In the Russian Federation, research is currently underway to determine regulatory values for a number of biochemical laboratory parameters in juvenile high-performance athletes [9, 10]. In this study, we aim to evaluate the bone metabolism status of healthy high-performance athletes under the age of 18 based on the levels of β-CrossLaps, osteocalcin, and P1NP in the blood serum.

MATERIALS AND METHODS

A single-center, cross-sectional study involved young athletes from the national teams of the Russian Federation who had underwent an in-depth medical examination at the Federal Scientific and Clinical Center for Children and Adolescents in the period from March 2021 to July 2023. A total of 383 young athletes aged 13–18 years participated in the study, including 248 girls and 135 boys; the average age was 15.2 [14.0; 16.1] years. All athletes were divided into groups according to gender and age. The male groups were as follows: 13.1–14.0 years old (n = 3), 14.1–15.0 years old (n = 11), 15.1–16.0 years old (n = 43), 16.1–17.0 years old (n = 42), and 17.1–18.0 years old (n = 36). The female groups were as follows: 13.1–14.0 years old

(*n* = 17), 14.1–15.0 years old (*n* = 51), 15.1–16.0 years old (*n* = 65), 16.1–17.0 years old (*n* = 59), and 17.1–18.0 years old (*n* = 56).

According to sexual maturity, athletes were distributed as follows: 5 (1.3%) athletes did not enter puberty, 17 (4.4%) — stage 2 sexual maturity, 57 (14.8%) athletes — stage 3, 174 (45.4%) athletes — stage 4 sexual maturity, the remaining 130 athletes had reached sexual maturity. The sexual maturity assessment was carried out according to the Tanner Scale (Sexual Maturity Rating, SMR) [11]. The key inclusion criteria were athletes from Russian national teams aged 13–18 years. The exclusion criteria were the presence of fractures during one year prior to inclusion in the study.

For clinical and laboratory tests, peripheral vein blood was collected in the morning after fasting. In all young athletes, the levels of osteocalcin (Roche, Switzerland), N-terminal propeptide human procollagen type 1/P1NP (Roche, Switzerland), and C-terminal telopeptide/β-CrossLaps (Roche, Switzerland) in blood serum (ng/mL) were evaluated. The β-CrossLaps assay was performed by electrochemiluminescence using a Cobase411 analyzer (Roche Diagnostics, Germany). The level of N-terminal propeptide human procollagen type 1 (P1NP) and osteocalcin was detected by enzyme-linked immunosorbent assay (ELISA). The P1NP level was assessed by reference intervals proposed by Chubb et al. [12]. The osteocalcin level was evaluated by reference intervals proposed by Bayer et al. [13]. The level of β-CrossLaps was calculated according to the reference intervals proposed by Crofton et al. [14].

Statistical data processing was performed using the Statistica 10.0 software package (StatSoft Inc.; USA). Since the parameters under study showed a non-normal distribution (according to the Kolmogorov–Smirnov test), all the data are presented as the median (M_e) of both the first and third quartiles [Q_1; Q_3]. To assess the statistical significance of the differences in quantification, the Mann–Whitney and Kruskall–Wallis tests were used, including with the Bonferroni adjustment. Qualitative characters are presented in proportions (%) with absolute values. To assess the differences between qualitative characters, contingency tables are charted with subsequent evaluation according to the Pearson's chi-squared test. The statistical significance of the differences was $p < 0.05$.

RESULTS

The levels of β-CrossLaps in athletes under the age of 18, depending on age and gender, were found to be statistically significantly higher in boys ($p < 0.01$) (Table 1). The established gender differences in the β-CrossLaps level are most likely to be associated with the larger bone mass accrual in boys compared to girls. The maximum values of β-CrossLaps in boys (2.27 [1.14; 3.45] ng/mL) and girls (1.55 [1.10; 2.02] ng/mL) are reached at the age of 13–14 years.

When assessing the β-CrossLaps levels in athletes under the age of 18, compared with the reference intervals proposed for children by Crofton et al. [14], significant differences were found, manifested in an increase in this parameter in athletes compared with its values in children in the general pediatric population, regardless of gender and age (Fig. 1).

Figure 1 shows that most of the individual β-CrossLaps values in both boys and girls exceeded the upper limit of the reference interval.

The maximum values of β-CrossLaps were determined in young athletes with stage 3 sexual maturity at the level of 2.31 [1.91; 3.4] ng/mL in boys and 1.98 [1.51; 2.31] ng/mL in girls (Table 2). Such changes are associated with active bone metabolism during peak growth and accrual of muscle tissue in adolescents.

Table 1. β-CrossLaps levels in athletes under the age of 18, depending on gender and age

Age, years	Boys			Girls			Statistical significance level, *p*
	Median [Q_1; Q_3]	Min	Max	Median [Q_1; Q_3]	Min	Max	
13.1–14.0	2.27 [1.14; 3.45] *n* = 3	0.330	5.300	1.55 [1.10; 2.02] *n* = 17	0.480	2.52	< 0.01
14.1–15.0	2.21 [1.64; 2.47] *n* = 11	0.510	4.450	1.420 [1.19; 1.68] *n* = 51	0.690	2.77	< 0.01
15.1–16.0	1.46 [1.11; 2.00] *n* = 43	0.520	3.080	1.10 [0.88; 1.40] *n* = 65	0.480	4.03	< 0.01
16.1–17.0	1.20 [0.68; 1.72] *n* = 42	0.320	4.140	0.92 [0.65; 1.09] *n* = 59	0.310	2.46	< 0.01
17.1–18.0	1.25 [0.87; 1.75] *n* = 36	0.320	2.660	0.86 [0.53; 1.09] *n* = 56	0.250	5.90	< 0.01

Table compiled by the authors based on their own data

Note: *n* — number of athletes.

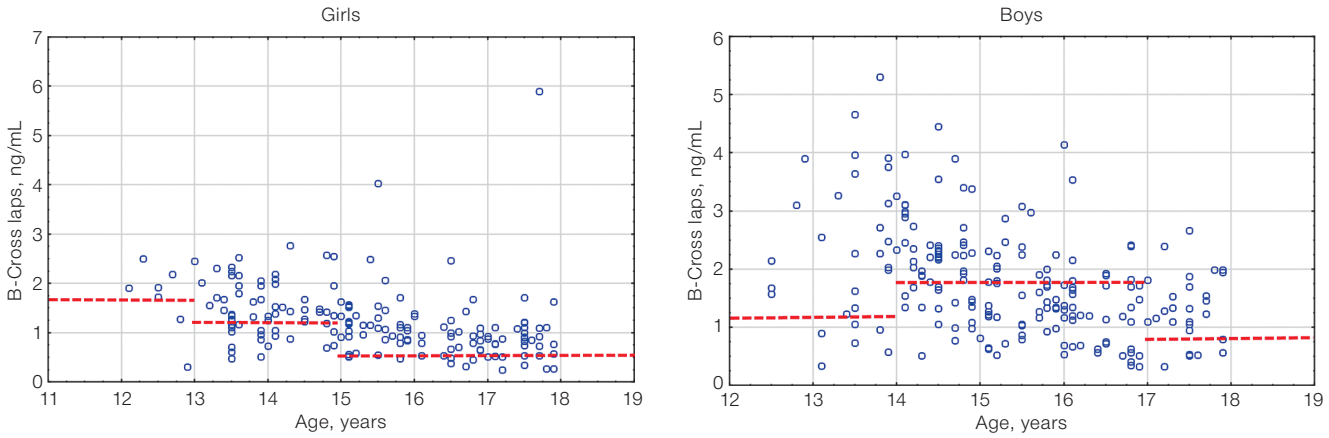

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Fig. 1. β-CrossLaps values in athletes under the age of 18 in comparison with the general pediatric reference intervals depending on age: the red dotted lines indicate the upper limit of the general pediatric reference intervals of β-CrossLaps according to [14] for boys and girls, taking into account age; the blue circles represent the individual values of β-CrossLaps for each athlete; *n* — number of athletes

When assessing osteocalcin levels in athletes under the age of 18, statistically significantly higher osteocalcin levels were found in boys, depending on gender, compared to girls in all age groups (Table 3). The maximum values of osteocalcin in boys (125 [89; 144] ng/mL) are revealed at the age of 14–15 years old; in girls (86 [62; 131] ng/mL) — at the age of 13–14 years, which is also due to the earlier onset of puberty in girls.

Osteocalcin levels in athletes under the age of 18 did not exceed the limits of the reference interval [13] (Fig. 2). As shown in Figure 2, most of the individual values of osteocalcin in both boys and girls remained within the reference interval.

When assessing the levels of P1NP in athletes under the age of 18, statistically significantly higher levels of P1NP were found in boys, depending on gender, compared to girls in all age groups (Table 4). The maximum values of P1NP were determined at the age of 13–14 years, in both boys (767.8 [148.1; 1142.4] ng/mL) and girls (450.5 [268.6; 569.3] ng/mL).

When assessing the levels of P1NP in athletes under the age of 18, compared to the reference intervals proposed by Chubb et al. for children [12], no gender differences were found (Fig. 3). As shown in Figure 3, most of the individual P1NP values in both boys and girls did not exceed the reference values.

When assessing the levels of osteocalcin and P1NP depending on the stage of sexual maturity according to the Tanner Scale, the maximum values of osteocalcin (102 [77; 131] ng/mL) were revealed in athletes with stage 3 sexual maturity, and the maximum level of P1NP (642.3 [537.9; 789.3] ng/mL) — in athletes with stage 2 sexual maturity (Table. 5). These data correlate with the growth rate peak value in adolescents.

DISCUSSION

β-CrossLaps is the most informative marker of bone resorption that indicates the activity of osteoclasts. This marker is widely used in clinical pediatric practice and is included in medical examination programs of young athletes. Permanently elevated β-CrossLaps levels in adult athletes indicate their prolonged exposure to high-intensity loads and inconsistency with their overall physical fitness level, which can lead to chronic overexertion or microtrauma, disrupting the structure and function of bone tissue [15]. Assessment of β-CrossLaps levels in young high-performance athletes revealed an increase in this

Table 2. β-CrossLaps levels in athletes under the age of 18, depending on gender and sexual maturity stage

Sexual maturity rating (Tanner stages)	1	2	3	4	5
Boys	1.57 [1.34; 1.74] *n* = 1	2.14 [1.97; 2.51] *n* = 11	2.31 [1.91; 3.4] *n* = 17	2.14 [1.64; 2.65] *n* = 56	1.45 [1.23; 1.88] *n* = 50
Girls	1.29 *n* = 4	1.87 [1.78; 2.30] *n* = 6	1.98 [1.51; 2.31] *n* = 40	1.33 [0.96; 1.56] *n* = 118	1.22 [0.98; 1.29] *n* = 80

Table compiled by the authors based on their own data

Note: *n* — number of athletes.

Table 3. Osteocalcin levels in athletes under the age of 18, depending on gender and age

Age, years	Boys			Girls			Statistical significance level, *p*
	Median [Q_1; Q_3]	Min	Max	Median [Q_1; Q_3]	Min	Max	
13.1–14.0	113 [88; 155] *n* = 3	88.0	155.0	86 [62; 131] *n* = 17	36.0	229.0	–
14.1–15.0	125 [89; 144] *n* = 11	77.0	201.0	68 [49; 98] *n* = 51	17.0	145.0	0.013
15.1–16.0	78 [63; 106] *n* = 43	13.0	190.0	58 [43; 71] *n* = 65	28.0	137.0	0.034
16.1–17.0	68 [53; 90] *n* = 42	28.0	174.0	44 [35; 61] *n* = 59	24.0	110.0	0.027
17.1–18.0	51 [42; 63] *n* = 36	10.0	110.0	36 [31; 49] *n* = 56	23.0	146.9	0.021

Table compiled by the authors based on their own data

Note: *n* — number of athletes; "–" — non-significant statistical results.

parameter in all age groups, particularly in 14–15-year-old athletes. The average values of β-CrossLaps in athletes are two- or threefold higher than the standard values for people with a daily level of physical activity [15]. The authors explain the increased level of this marker by the specific nature of anabolic processes in juvenile athletes. Establishing a correlation with the type of athletic discipline, Klyuchnikov et al. found the maximum values of β-CrossLaps in athletes engaged in game sports, with the average values of β-CrossLaps in boys being higher than those in girls [15].

The results of our work also demonstrate the previously described gender differences and the fact that the β-CrossLaps level in young athletes is higher than that in the general pediatric population.The maximum values of β-CrossLaps in young athletes are recorded at the 2nd–3rd stage of puberty, which, according to the literature [14], coincides with the peak height velocity and is associated with the peak bone mass gain. Thus, the increased activity of bone resorption detected in young athletes compared with their peers with normal physical activity may be due to the intensity and nature of physical exertion.

Osteocalcin is a non-collagen protein of the bone matrix, synthesized by osteoblasts. This parameter reflects the osteosynthesis activity. Osteocalcin levels gradually increase in childhood, reaching their maximum values in puberty. The osteocalcin concentration in children correlates with the height velocity and increases progressively during puberty, regardless of gender. The maximum levels of this biomarker are recorded at the age of 13–14 years, ranging 25–241 ng/mL [13].

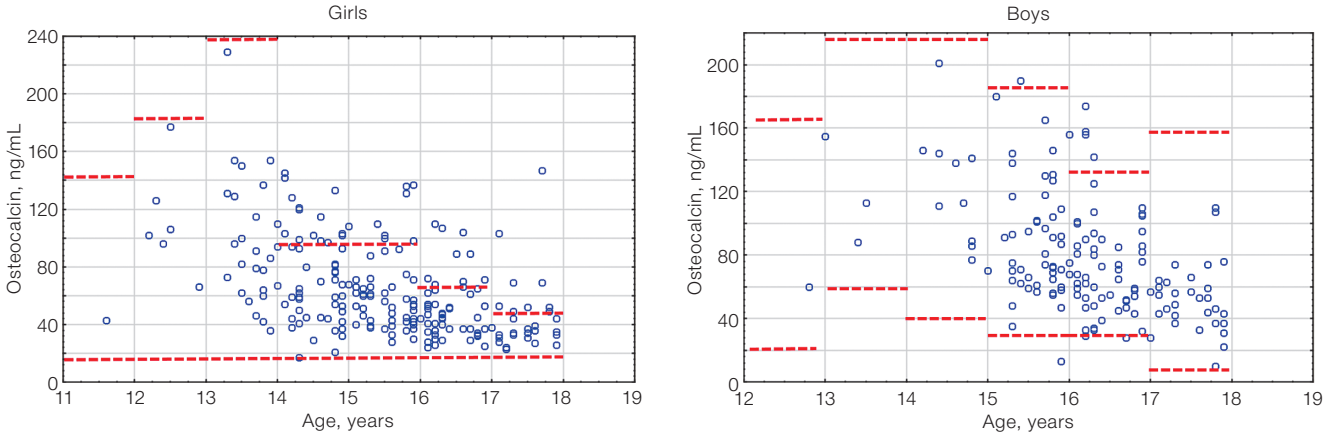

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Fig. 2. Osteocalcin levels in athletes under the age of 18 compared to the general pediatric reference intervals depending on gender: the red dotted lines indicate the upper and lower boundaries of the general pediatric reference intervals [13] for boys and girls, taking into account age; the blue circles represent individual osteocalcin values for each athlete; *n* — number of athletes; "–" — non-significant statistical results

Table 4. P1NP levels in athletes under the age of 18, depending on gender and age

Age, years	Boys			Girls			Statistical significance level, *p*
	Median [Q_1; Q_3]	Min	Max	Median [Q_1; Q_3]	Min	Max	
13.1–14.0	767.8 [148.1;1142.4] *n* = 3	148.1	1142.4	450.5 [268.6; 569.3] *n* = 17	128.2	1324.0	–
14.1–15.0	689.9 [548.4;727.7] *n* = 11	446.0	1398.0	250.2 [209.0;599.6] *n* = 51	75.3	1053.0	0.023
15.1–16.0	425.1 [300.7;662.2] *n* = 43	127.1	1298.2	239.0 [167.1;369.0] *n* = 65	85.8	838.1	0.034
16.1–17.0	288.8 [219.4;473.9] *n* = 42	115.2	1155.0	164.5 [119.6;228.7] *n* = 59	78.9	611.1	0.017
17.1–18.0	227.0 [182.4;278.3] *n* = 36	95.1	680.2	138.0 [106.3;188.8] *n* = 56	39.0	335.1	0.022

Table compiled by the authors based on their own data

Note: *n* — number of athletes.

Table 5. Osteocalcin and P1NP levels in athletes under the age of 18, depending on sexual maturity rating

Sexual maturity rating (Tanner stages)	1	2	3	4	5
Number of athletes, *n*	5	17	57	174	130
Osteocalcin	60 [46; 88]	97 [89; 121]	102 [77; 131]	62 [44; 81]	49 [37; 65]
P1NP	296.1 [153.1;459.7]	642.3 [537.9;789.3]	605.3 [432.1;769.7]	243.6 [176.2;408.1]	200.9 [149.4;270.0]

Table compiled by the authors based on their own data

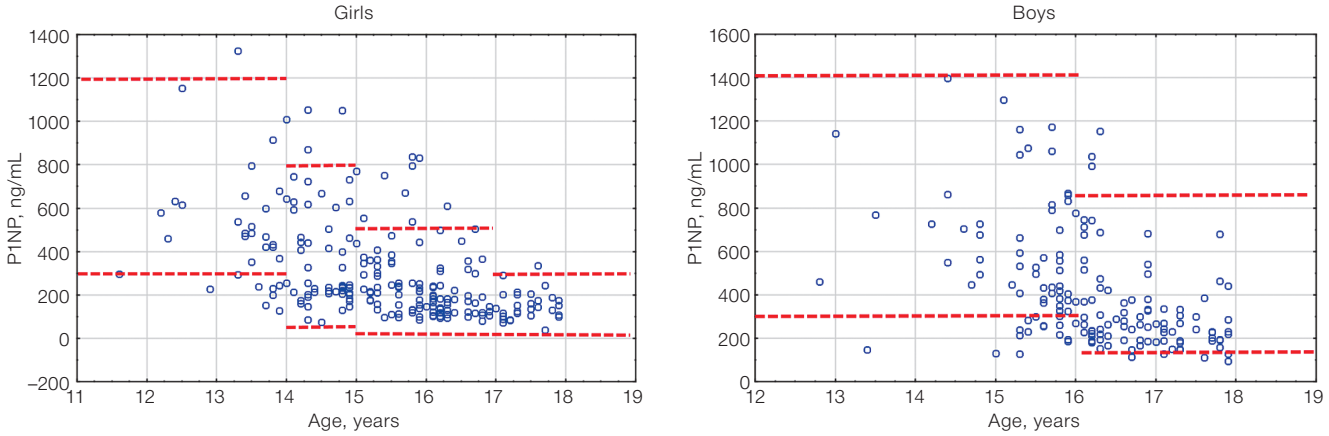

Figure prepared by the authors based on their own data

Fig. 3. P1NP values in athletes under the age of 18 compared with the general pediatric reference intervals depending on gender: the red dotted lines indicate the upper and lower limits of the general pediatric reference intervals [12] for boys and girls, taking into account age; the blue circles represent individual P1NP values for each athlete; *n* — number of athletes

Our study has demonstrated that the maximum level of osteocalcin is recorded in girls in the age group of 13–14 years, in boys — in the age group of 14–15 years. Osteocalcin values increase with the progression of sexual development from stage 1 to stage 2, followed by a decrease by the end of puberty. In children and adolescents, more than 90% of the synthesized osteocalcin is incorporated into the bone matrix, with only its small part circulating in the circulatory system. In addition, the level of osteocalcin is subject to pronounced daily fluctuations; therefore, the study should be conducted in the morning. Osteocalcin levels in young athletes are higher than those in adults [5].

The N-terminal propeptide of human procollagen type 1 is a marker of bone matrix formation. The variability and higher levels of P1NP in childhood are due to the active processes of child growth and development. The acceleration of height velocity in early childhood and during sexual maturation is accompanied by a significant increase in the P1NP level. The maximum values of this biomarker in boys are recorded in the first year of life, reaching the values of 3000 ng/mL, with a gradual decrease to 950 ng/mL by the age of 11. In the 11–16-year group, a repeated increase in the P1NP level to 1400 ng/mL was observed. The lower limit of P1NP in this age group in boys is 300 ng/mL. In girls, the maximum values of P1NP are also recorded in the first year of life (600–3000 ng/mL) with a gradual decrease by the age of 9 years. From 9 to 14 years of age, serum P1NP levels continue to rise (the standard range is 300–1200 ng/mL) [12]. The described gender-specific features of P1NP secretion in childhood are due to the different timing of the onset of sexual maturation in boys and girls. Thus, the maximum values of P1NP in boys are recorded at stage 3 sexual maturity according to the Tanner scale; in girls — at stage 2 of sexual maturity according to the Tanner Scale [12].

Our study has also demonstrated that the maximum levels of P1NP in young athletes are observed at the age of 13–15 years, with a further gradual decrease towards the end of sexual maturation.

Thus, the levels of bone synthesis markers in young athletes correlate with the reference intervals for the general pediatric population, with their variability in childhood being due to increased metabolism in bone tissue during the period of active growth.

An important limitation of this study is the lack of information at the time of blood sampling about the intake of colecalciferol and other biologically active additives that affect bone metabolism. In addition, the study did not evaluate the discipline-related effect on the studied bone metabolism markers due to the limited sample size of athletes. Nevertheless, the obtained clinical results demonstrate a tendency to higher values of osteocalcin and P1NP in athletes engaged in complex coordination sports. This aspect requires additional elucidation, which can be of practical importance for developing an individualized approach to interpretation of laboratory parameters.

CONCLUSION

The level of β-CrossLaps, the main marker of bone resorption, in young high-performance athletes was found to be significantly higher than the population norms for children and adolescents with a normal level of physical activity. When assessing the β-CrossLaps level in athletes under the age of 18, reference values based on gender and sexual maturity stage should be used. The β-CrossLaps levels in girls are statistically significantly lower than those in boys, which may be due to larger bone and muscle mass in males.

The reference values of osteocalcin and P1NP in young athletes correlate with the values recorded in the general pediatric population. However, when assessing osteocalcin and P1NP levels in athletes under the age of 18, reference values should be adjusted for the stage of sexual maturity, since these bone remodeling markers are characterized by a physiological increase against the background of accelerated height velocity during sexual maturation.

The data obtained can be used when interpreting the results of an in-depth medical examination of athletes from Russian national sports teams to identify bone remodeling disorders and form individual prevention and correction programs within the framework of medical and biological support for high-performance sports.

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DEVELOPMENT OF PREDICTIVE MATHEMATICAL MODELS FOR PHYSICAL PERFORMANCE PARAMETERS IN SPORTS AND SPORTS MEDICINE

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Introduction. Predictive modeling in healthcare is a rapidly evolving field of scientific knowledge at the intersection of information technology and medicine. In sports medicine, the importance of accurate forecasting of physical performance parameters in response to changing environmental conditions cannot be overstated. For athletes, such information provides a crucial competitive advantage before major competitions.
Objective. Development of methods and approaches to analyze clinical data obtained through comprehensive medical examinations of athletes.
Materials and methods. An analysis of anonymized medical data from comprehensive medical examinations was conducted for 6222 world-class athletes (3792 males and 2430 females) with a mean age of 23.3 ± 5.1 years. The data were stratified by sex and according to sports categories: cyclic sports (1376 athletes, including 861 males and 515 females); complex coordination sports (1342 athletes, including 761 males and 581 females); team sports (1618 athletes, including 980 males and 638 females); and combat sports (1886 athletes, including 1190 males and 696 females). The analysis included both clinical data on the presence (or absence) of pathological conditions identified during specialist medical examinations and physiological parameters from bicycle ergometer stress testing. Statistical analysis was performed using the StatTech v. 4.6.0 software (StatTech, Russia).
Results. Using regression analysis, statistically significant ($p < 0.001$) predictive models for a set of physical performance parameters were developed, which revealed over 40 associations with clinical diagnoses made by medical specialists. The strongest correlations were observed between physical performance indicators and dental diagnoses. Future research will focus on creating a mathematical model to predict performance decline in world-class athletes, based on an analysis of disease development risk factors.
Conclusions. The developed and implemented approaches for analyzing clinical data from comprehensive medical examinations of world-class athletes enabled the creation of effective predictive mathematical models of physical performance parameters using linear regression methodology, while accounting for the presence/absence of identified diagnoses. The proposed models provide a comprehensive assessment of athletes' functional status, thus allowing accurate prediction of physical performance levels and optimization of professional training by minimizing risks of overtraining and sports-related injuries.

Keywords: high-performance sports; mathematical model; physical performance parameters; pathological condition; regression analysis

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Compliance with ethical principles: the study was approved by the local Ethics Committee of the Burnasyan Federal Medical Biophysical Center (protocol No. 121 dated 23.01.2025). All participants signed a voluntary consent form for the use of their anonymized medical data in scientific research.

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РАЗРАБОТКА ПРОГНОСТИЧЕСКИХ МАТЕМАТИЧЕСКИХ МОДЕЛЕЙ ПАРАМЕТРОВ ФИЗИЧЕСКОЙ РАБОТОСПОСОБНОСТИ В СПОРТЕ И СПОРТИВНОЙ МЕДИЦИНЕ

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Введение. Прогностическое моделирование в здравоохранении — новая развивающаяся отрасль научного знания, находящаяся на стыке информационных технологий и медицины. Для спортивной медицины наличие точного прогноза параметров физической работоспособности в ответ на изменяющиеся условия внешней среды сложно переоценить, а для спортсмена подобная информация даст необходимое конкурентное преимущество при проведении ответственных соревнований.
Цель. Разработка методов и подходов к анализу клинических данных углубленного медицинского обследования (УМО) спортсменов.
Материалы и методы. Проведен анализ обезличенных медицинских данных результатов УМО для 6222 спортсменов высокого класса (3792 мужчины и 2430 женщин) (средний возраст 23,3 ± 5,1 года). Данные были распределены по полу и в соответствии с группами видов спорта: циклические виды спорта (1376 спортсменов, из них 861 мужчина и 515 женщин); сложнокоординационные виды спорта (1342 спортсмена, из них 761 мужчина и 581 женщина); игровые виды спорта (1618 спортсменов, из них 980 мужчин и 638 женщин) и спортивные единоборства (1886 спортсменов, из них 1190 мужчин и 696 женщин). Анализу подверглись как клинические данные по наличию (отсутствию) нозологических единиц, выявленных в ходе осмотров врачами-специалистами,

так и физиологические показатели нагрузочного тестирования на велоэргометре. Статистический анализ проведен с использованием программы StatTech v. 4.6.0 (разработчик — ООО «Статтех», Россия).

Результаты. В результате на основе метода регрессионного анализа были построены достоверные ($p < 0,001$) прогностические модели группы параметров физической работоспособности, которые выявили наличие более 40 связей с клиническими диагнозами врачей-специалистов. Больше всего взаимосвязей было зафиксировано между группой показателей физической работоспособности и проставленными диагнозами стоматолога. Дальнейшая работа будет направлена на разработку математической модели прогнозирования снижения результативности у спортсменов спорта высших достижений, основанной на анализе рисков развития заболеваний.

Выводы. Разработанные и примененные подходы к анализу клинических данных углубленного медицинского обследования спортсменов высокого класса позволили, применяя метод линейной регрессии, создать эффективные прогностические математические модели параметров физической работоспособности с учетом наличия/отсутствия выявленного диагноза. Предложенные модели обеспечивают комплексную оценку функционального состояния спортсменов, что способствует более точному прогнозированию уровня физической работоспособности и позволяет оптимизировать профессиональную деятельность, минимизируя риски перетренированности и травматизма.

Ключевые слова: спорт высших достижений; математическая модель; параметры физической работоспособности; нозологическая единица; регрессионный анализ

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INTRODUCTION

The application of mathematical models to predict and evaluate physiological parameters in professional athletes is closely related to both advancements in sports science and analytical methods.

In the field of sports performance analysis and forecasting, modern statistical modeling techniques are increasingly finding application, driving the transformation of research methodologies worldwide. While sports science has traditionally relied on conventional statistical approaches, recent innovations have introduced more sophisticated tools such as machine learning algorithms and hierarchical modeling. These advanced techniques enable researchers to identify complex relationships within both medical and athletic data, leading to deeper insights into the predictors of performance decline and the optimization of training strategies [1–6].

Previous research in this domain has primarily focused on understanding individual and collective trends in general and sport-specific physical performance metrics. For instance, studies on running performance have explored three key areas: (1) physiological determinants of world-record achievements; (2) development of equivalent scoring and race outcome prediction systems; and (3) modeling individual physiological parameters in track and field athletes [7–9]. Despite these efforts, there is a lack of comprehensive knowledge-based models capable of integrating and comparing all the above aspects. This determines the relevance of developing versatile, personalized, and accessible mathematical models for predicting physical performance parameters in sports.

Recent achievements in the integration and analysis of big data have enhanced the accuracy of performance predictions. In one study, the researchers used an online database of the performance of British athletes (1954–2013) to propose a simplified model capturing key performance characteristics while maintaining empirical validity. This model demonstrated a remarkably low mean prediction error for specific athletic outcomes, marking an important step toward unifying performance understanding through data analytics [10, 11].

A review of literature on predictive mathematical models in sports medicine reveals that most studies focus either on injury risk prediction across various sports or on estimating the probability of achieving target performance outcomes [12–15]. However, no studies examining potential correlations between diagnosed medical conditions in world-class athletes and their predicted impact on physical performance decline have been found.

With advances in neural networks and machine learning, researchers — including international collaborations — are now combining efforts to study the interplay of physiological and psychological factors affecting athletic performance. The goal is to optimize training regimens through predictive models capable of accounting for the dynamic nature of sports performance and its relationship with athletes' physiological processes.

The present study is aimed at developing methods for analyzing clinical data from comprehensive medical examinations (CME) of athletes.

MATERIALS AND METHODS

This study analyzed and mathematically processed anonymized clinical and instrumental examination data derived from medical records of world-class athletes who underwent comprehensive medical examinations (CME) in the Burnasyan Federal Medical Biophysical Center in 2019–2023. The dataset included clinical information from 6222 athletes (3792 males and 2430 females, mean age 23.3 ± 5.1 years).

The data were stratified by sex and sport categories: cyclic sports (1376 athletes, including 861 males and 515 females); complex coordination sports (1342 athletes, including 761 males and 581 females); team sports (1618 athletes, including 980 males and 638 females); and combat sports (1886 athletes, including 1190 males and 696 females).

The article presents data on male athletes from cyclic (n = 861) and team sports (n = 980). These cohorts were selected as those capable of providing the most representative and homogeneous samples for robust statistical analysis and reliable mathematical modeling. Data from other sports categories were utilized for preliminary screening and selection purposes.

During model development, the following inclusion criteria were applied:

- parameters had to reflect the key indicators of physical condition and functional capacity (morphofunctional status, aerobic/anaerobic capabilities, etc.);
- data were collected within homogeneous athlete groups (matched by sex, age, sports category, and skill level);
- measurements were obtained using standardized protocols (with uniform CME procedures for exercise testing).

The exclusion criteria comprised incomplete, erroneous, or anomalous parameters that could distort modeling results.

Thus, the following baseline parameters for mathematical modeling were used: age (years); weight (kg); height (cm); oxygen consumption at AT (anaerobic threshold) level ($VO_{2\ AT}$, mL/min/kg); peak oxygen consumption during exercise testing ($VO_{2\ peak}$, mL/min/kg); respiratory exchange ratio (R, relative units); resting heart rate (HR_{rest}, bpm); heart rate at aerobic threshold level (HR_{AerT}, bpm); heart rate at AT level (HR_{AT}, bpm); peak exercise heart rate (HR_{peak}, bpm); heart rate at 3 min of recovery (HR_{3min}, bpm); power output at anaerobic threshold level (Pwr_{AT}, W); peak power output during testing (Pwr_{peak}, W); relative power output at anaerobic threshold level (Pwr_{AT}/weight, W/kg); relative peak power output during testing (Pwr_{peak}/weight, W/kg). In each sports category, the statistical dataset of the studied parameters was combinatorially grouped by qualitative presence (1)/absence (0) of pathological conditions diagnosed by the following medical specialists: gastroenterologist, dermatovenerologist, cardiologist, neurologist, otolaryngologist, ophthalmologist, dentist, orthopedic traumatologist, and endocrinologist.

All quantitative parameters in the modeling are presented as: X_M — sex (0 — female, 1 — male); X_{age} — age; X_{weight} — weight; X_{height} — height; X_{gastro} — pathological condition by gastroenterologist (0 — no, 1 — yes); X_{derm} — pathological condition by dermatovenerologist (0 — no, 1 — yes); X_{cardio} — pathological condition by cardiologist (0 — no, 1 — yes); X_{neuro} — pathological condition by neurologist (0 — no, 1 — yes); X_{oto} — pathological condition by otolaryngologist (0 — no, 1 — yes); X_{ophth} — pathological condition by ophthalmologist (0 — no, 1 — yes); X_{dent} — pathological condition by dentist (0 — no, 1 — yes); X_{trauma} — pathological condition by orthopedic traumatologist (0 — no, 1 — yes); X_{endo} — pathological condition by endocrinologist (0 — no, 1 — yes); $X_{VO2\ AT}$ — $V(O_2)$ at AT level; $X_{VO2\ peak}$ — $V(O_2)$ at peak; X_R — respiratory coefficient; $X_{HR\ rest}$ — HR at rest; $X_{HR\ AerT}$ — HR at AerT; $X_{HR\ AT}$ — HR at AT; $X_{HR\ peak}$ — HR at peak; $X_{HR\ 3min}$ — HR at 3-min recovery; $X_{PWR\ AT}$ — power at AT; $X_{PWR\ peak}$ — power at peak; $X_{PWR\ AT/weight}$ — power at AT divided by weight; $X_{PWR\ peak/weight}$ — power at peak divided by weight.

Statistical analysis was performed using the StatTech v. 4.6.0 software (StatTech, Russia). The linear regression method was used to examine the dataset structure and establish relationships between its parameters. Mathematical models were developed to describe dependencies between the group of quantitative physical performance indicators and the presence/absence of pathological conditions identified by physicians during in-depth medical examinations, for each sport category and sex. Regression equation coefficients were determined using the least squares method with Cramer's formulas.

RESULTS AND DISCUSSION

During data preparation for linear regression modeling, the relevant CME results of athletes were compiled to ensure the required precision. Incomplete, erroneous, or anomalous values were excluded, along with non-informative features. Table 1 presents the general characteristics of male athletes from cyclic and team sports included in the final sample.

Among the exercise testing parameters characterizing the overall physical performance (items 4–15 in Table 1), the most physiologically relevant indicators for sports medicine applications are those of gas exchange: $VO_{2\ AT}$, $VO_{2\ peak}$, respiratory exchange ratio, as well as the direct measure of achieved power output at anaerobic threshold.

The models presented below describe general relationships (and interdependencies) between the selected physical performance metrics (*Y* value in the formula) and all other parameters, including the presence/absence of pathological conditions during examination (*X* values in the formula).

The observed relationship for oxygen consumption at anaerobic threshold $VO_{2\ AT}$ (1 — cyclic sports, 2 — team sports) is described by the following linear regression equations:

$$Y_{VO2\ AT} = -5.313 - 0.424 \times X_{neuro} - 0.369 \times X_{dent} + 0.921 \times X_{endo} + 0.058 \times X_{weight} + 0.479 \times X_{VO2\ peak} - \\ - 0.024 \times X_{HR\ rest} + 0.029 \times X_{HR\ AT} + 0.038 \times X_{PWR\ AT} - 0.047 \times X_{PWR\ peak} + 5.924 \times X_{PWR\ AT/weight}, \quad (1)$$

$$Y_{VO2\ AT} = -0.301 + 0.572 \times X_{M} + 0.576 \times X_{VO2\ peak} - 0.022 \times X_{HR\ rest} + 0.072 \times X_{HR\ AT} - 0.033 \times X_{HR\ peak} - \\ - 0.012 \times X_{HR\ 3min} - 0.004 \times X_{PWR\ peak} + 7.671 \times X_{PWR\ AT/weight} - 3.725 \times X_{PWR\ peak/weight}. \quad (2)$$

The obtained regression models for oxygen consumption at anaerobic threshold ($VO_{2\ AT}$) demonstrated the following characteristics:

1. The multiple correlation coefficient was $R_{xy} = 0.965$ for cyclic sports and $R_{xy} = 0.948$ for team sports, indicating a highly strong relationship according to the Chaddock scale;

2. The coefficient of multiple determination was $R^2 \approx (0.965)^2 = 0.931$ for cyclic sports and $R^2 \approx (0.948)^2 = 0.899$ for team sports. These models can predict $VO_{2\ AT}$ values with high accuracy: they explain 93.1% of observed variance in cyclic sports and 89.9% in team sports. The models were statistically significant ($p < 0.001$).

After accounting for interdependencies among physical performance parameters, negative associations were found between $VO_{2\ AT}$ and neurological/dental pathological conditions, while a positive association was observed with endocrine disorders.

The relationship for peak oxygen consumption ($VO_{2\ peak}$) (3 — cyclic sports, 4 — team sports) is described by the following linear regression equation:

$$Y_{VO2\ peak} = 5.920 + 0.737 \times X_{M} - 1.417 \times X_{endo} + 0.865 \times X_{VO2\ AT} - 0.050 \times X_{HR\ AT} + \\ + 0.036 \times X_{HR\ peak} - 6.026 \times X_{PWR\ AT/weight} + 6.668 \times X_{PWR\ peak/weight}, \quad (3)$$

$$Y_{VO2\ peak} = 5.743 + 5.743 \times X_{M} - 0.091 \times X_{age} + 0.823 \times X_{VO2\ AT} - 6.022 \times X_{R} + + 0.023 \times X_{HR\ rest} - \\ - 0.076 \times X_{HR\ AT} + 0.048 \times X_{HR\ peak} - 6.090 \times X_{PWR\ AT/weight} + + 8.022 \times X_{PWR\ peak/weight}. \quad (4)$$

The key characteristics of the developed models:

1. The multiple correlation coefficient was $R_{xy} = 0.933$ for cyclic sports and $R_{xy} = 0.919$ for team sports, indicating a highly strong association according to the Chaddock scale.

2. The coefficient of determination reached $R^2 \approx (0.933)^2 = 0.871$ for cyclic sports and $R^2 \approx (0.919)^2 = 0.844$ for team sports.

The models demonstrate high predictive accuracy for $VO_{2\ peak}$ values, explaining 87.1% of the observed variance in cyclic sports and 84.4% in team sports. All models showed statistical significance ($p < 0.001$).

After analyzing interdependencies among physical performance parameters, a negative association between $VO_{2\ peak}$ and endocrine disorders was observed.

The relationship between the respiratory exchange ratio (*R*) (5 — cyclic sports, 6 — team sports) and quantitative factors is described by the following linear regression equation:

$$Y_{R} = 1.436 - 0.013 \times X_{M} + 0.013 \times X_{dent} - 0.002 \times X_{height} - 0.002 \times X_{weight} - 0.0001 \times X_{HR\ rest} + 0.0001 \times X_{HR\ AerT} - \\ - 0.001 \times X_{HR\ AT} + 0.001 \times X_{HR\ peak} + 0.001 \times X_{HR\ 3min} + 0.001 \times X_{PWR\ AT} - 0.167 \times X_{PWR\ AT/weight} + 0.107 \times X_{PWR\ peak/weight}, \quad (5)$$

$$Y_{R} = 0.388 + 0.388 \times X_{M} + 0.012 \times X_{ophth} + 0.009 \times X_{dent} + 0.002 \times X_{weight} - 0.002 \times X_{VO2peak} - 0.001 \times X_{HR\ AT} + \\ + 0.000 \times X_{HR\ 3min} + 0.001 \times X_{HR\ peak} - 0.001 \times X_{PWR\ AT} + 0.092 \times X_{PWR\ peak/weight}. \quad (6)$$

The characteristics of the developed models are as follows:

1. For cyclic sports, the multiple correlation coefficient was $R_{xy} = 0.830$, while for team sports it was $R_{xy} = 0.783$, indicating a strong association according to the Chaddock scale.

2. The coefficient of determination was $R^2 \approx (0.830)^2 = 0.689$ for cyclic sports and $R^2 \approx (0.783)^2 = 0.613$ for team sports.

The models demonstrate moderately high predictive accuracy for the respiratory exchange ratio (*R*), explaining 68.9% of the observed variance in cyclic sports and

Table 1. Descriptive statistics of quantitative variables included in the analysis

No.	Examined parameter	Median (mean) parameter value	Value range	
			min	max
Cyclic sports (n = 861)				
1	Age, years	21.00 [19.0–25.0]	18.00	26.00
2	Height, cm	180.00 [172.0–186.0]	152.00	207.00
3	Weight, kg	74.00 [65.0–83.0]	43.00	120.00
4	$VO_{2\ AT}$, mL/min/kg	42.68 ± 9.71 (42.16–43.19)	14.74	68.36
5	$VO_{2\ peak}$, mL/min/kg	49.97 [43.99–57.25]	2.52	92.93
6	*R,* relative units	1.16 [1.10–1.23]	0.89	1.55
7	HR_{rest}, bpm	76.00 [67.00–85.00]	40.00	126.00
8	HR_{AerT}, bpm	120.00 [108.00–132.00]	61.00	175.00
9	HR_{AT}, bpm	155.00 [144.00–165.00]	95.00	196.00
10	HR_{peak}, bpm	173.00 [164.00–181.00]	18.00	206.00
11	HR_{3min}, bpm	102.00 [92.00–114.00]	45.00	155.00
12	Pwr_{AT}, W	275.00 [225.00–340.00]	90.00	520.00
13	Pwr_{peak}, W	345.00 [285.00–420.00]	115.00	600.00
14	Pwr_{AT}/weight, W/kg	3.81 ± 0.90 (3.76–3.85)	0.00	6.34
15	Pwr_{peak}/weight, W/kg	4.69 [4.15–5.43]	1.42	7.72
Team sports (n = 980)				
1	Age, years	22.00 [19.00–26.00]	18.00	26.00
2	Height, cm	182.00 [173.00–191.00]	151.00	220.00
3	Weight, kg	80.00 [68.00–92.00]	47.00	126.00
4	$VO_{2\ AT}$, mL/min/kg	33.42 [28.92–38.27]	14.26	58.86
5	$VO_{2\ peak}$, mL/min/kg	41.25 ± 6.84 (40.92–41.58)	15.85	69.41
6	*R,* relative units	1.14 [1.09–1.19]	0.90	1.52
7	HR_{rest}, bpm	79.00 [71.00–86.00]	44.00	142.00
8	HR_{AerT}, bpm	117.00 [107.00–128.00]	67.00	177.00
9	HR_{AT}, bpm	149.00 [137.00–159.00]	91.00	199.00
10	HR_{peak}, bpm	168.00 [159.00–176.00]	65.00	202.00
11	HR_{3min}, bpm	103.00 [93.00–112.00]	29.00	173.00
12	Pwr_{AT}, W	235.00 [195.00–285.00]	80.00	470.00
13	Pwr_{peak}, W	310.00 [245.00–365.00]	130.00	525.00
14	Pwr_{AT}/weight, W/kg	2.99 [2.57–3.44]	0.00	5.27
15	Pwr_{peak}/weight, W/kg	3.85 ± 0.63 (3.82–3.89)	1.91	6.18

Table prepared by the author using her own data

Note: $VO_{2\ AT}$, $VO_{2\ peak}$, Pwr_{AT}/weight, Pwr_{Peak}/weight are presented as mean ± standard error of the mean (M ± SEM); all other parameters are presented as median (M_e) with lower and upper quartiles Q [25–75%]; $VO_{2\ AT}$ — oxygen consumption at anaerobic threshold level, $VO_{2\ peak}$ — oxygen consumption at maximal exercise testing stage, R — respiratory exchange ratio, HR_{rest} — heart rate at rest (pre-exercise), HR_{AerT} — heart rate at aerobic threshold level, HR_{AT} — heart rate at anaerobic threshold level, HR_{peak} — peak heart rate during exercise, HR_{3min} — heart rate at 3 min of recovery, Pwr_{AT} — power output at anaerobic threshold level, Pwr_{Peak} — relative maximal power output during testing, Pwr_{AT}/weight — relative power output at anaerobic threshold per body weight, Pwr_{Peak}/weight — relative maximal power output per body weight during testing.

61.3% in team sports. All models were statistically significant ($p < 0.001$).

After controlling for interdependencies among physical performance parameters, positive associations were identified between the respiratory exchange ratio (R) and ophthalmological/dental pathological conditions.

The relationship between power output at anaerobic threshold (Pwr_{AT}) (7 — cyclic sports, 8 — team sports) and quantitative factors is described by the following linear regression equation:

$$Y_{PWR\ AT} = -62.319 + 0.115 \times X_{height} + 0.274 \times X_{weight} + 0.167 \times X_{VO2\ AT} + 17.519 \times X_{R} - 0.092 \times X_{HR\ AerT} + \\ + 0.153 \times X_{HR\ AT} - 0.069 \times X_{HR\ peak} + 0.724 \times X_{PWR\ peak} + 74.556 \times X_{PWR\ AT/weight} - 55.694 \times X_{PWR\ peak/weight}, \quad (7)$$

$$Y_{PWR\ AT} = -49.907 + 1.485 \times X_{neuro} + 1.185 \times X_{офт} - 0.102 \times X_{age} + 0.089 \times X_{height} + + 0.434 \times X_{weight} + \\ + 0.154 \times X_{HR\ AT} - 0.159 \times X_{HR\ peak} + 76.528 \times X_{PWR\ AT/weight} + 0.652 \times X_{PWR\ peak} - 48.533 \times X_{PWR\ peak/weight}. \quad (8)$$

The characteristics of the developed models are as follows:

1. The multiple correlation coefficient was $R_{xy} = 0.996$ for cyclic sports and $R_{xy} = 0.994$ for team sports, indicating an exceptionally strong association according to the Chaddock scale.

2. The coefficient of determination was $R^2 \approx (0.996)^2 = 0.993$ for cyclic sports and $R^2 \approx (0.994)^2 = 0.988$ for team sports. The models demonstrate extremely high predictive accuracy for power output at anaerobic threshold (Pwr_{AT}), explaining 99.3% of the observed variance in cyclic sports and 98.8% in team sports. All models were statistically significant ($p < 0.001$).

After controlling for interdependencies among physical performance parameters, positive associations were identified between Pwr_{AT} and neurological/ophthalmological pathological conditions.

In a similar manner, clinical data from 6222 athletes (accounting for gender and sport category differences) were processed. After developing 12 separate models for each sports category, significant relationships (both

Table 2. Statistically significant associations between physical performance parameters and clinically diagnosed medical conditions

Parameter / Medical specialist	$VO_{2\ AT}$	$VO_{2\ peak}$	R	HR_{rest}	HR_{AerT}	HR_{AT}	HR_{peak}	HR_{3min}	Pwr_{AT}	Pwr_{peak}	Pwr_{AT}/weight	Pwr_{peak}/weight
Gastroenterologist			1	1	1				1	1		1
Dermatovenerologist	1	1		1					1			
Cardiologist			2		1							
Neurologist	1								1		1	2
Otolaryngologist				1				1				
Ophthalmologist			2	1	2				1		1	1
Dentist	1		2	1	1	1		1	1	1	1	1
Orthopedic traumatologist					1			1				
Endocrinologist	2	2			1							

Table prepared by the author using her own data

Note: "1" — relationship between the relevant physical performance indicators and the presence of specialist-diagnosed conditions in one sport category; "2" — relationship between the relevant physical performance indicators and the presence of specialist-diagnosed conditions in two sports categories simultaneously; $VO_{2\ AT}$ — oxygen consumption at anaerobic threshold level, $VO_{2\ peak}$ — oxygen consumption at maximal exercise testing stage, R — respiratory exchange ratio, HR_{rest} — heart rate at rest (pre-exercise), HR_{AerT} — heart rate at aerobic threshold level, HR_{AT} — heart rate at anaerobic threshold level, HR_{peak} — heart rate at peak exercise, HR_{3min} — heart rate at 3 minutes of recovery, Pwr_{AT} — power output at anaerobic threshold level, Pwr_{Peak} — relative maximal power output during testing, Pwr_{AT}/weight — relative power output at anaerobic threshold normalized to body weight, Pwr_{Peak}/weight — relative maximal power output normalized to body weight during testing.

positive and negative) between the studied parameters and the presence/absence of diagnosed pathological conditions were quantified.

The total number of significant associations between physical performance parameters and the presence/absence of documented pathological conditions across all studied athlete groups reached 46 (Table 2).

Out of 46 established dependencies, the strongest correlations were found with ophthalmologist-diagnosed conditions (8 confirmed relationships) and dentist-diagnosed conditions (11 confirmed relationships). The developed mathematical models for predicting physical performance parameters confirmed a strong relationship between the probability of achieving planned training results or performance in major competitions and the presence of specialist-diagnosed conditions identified during the comprehensive medical examinations of athletes.

CONCLUSION

In this study, effective predictive mathematical models of physical performance parameters, accounting for the presence/absence of diagnosed conditions, have been developed using clinical data from comprehensive medical examinations of world-class athletes and linear regression methods. The developed models can be used to carry out a comprehensive assessment of athletes' functional status, thus facilitating a more accurate prediction of physical performance levels and optimization of professional activities while minimizing risks of overtraining and injuries.

Thus, the study results contribute to the development of sports medicine and provide a scientific basis for decision making in the field of athlete preparation and medical support. This will subsequently lead to changes in sports physicians' approaches to interpreting the results of comprehensive medical examinations.

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INTEGRATION PROSPECTS FOR THE MULTIPLEX PHOSPHORESCENCE IMMUNOASSAY OF POOLED DRY URINE SAMPLES INTO SCREENING EXAMINATIONS IN DISPENSARY DRUG CONTROL

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Introduction. The worsening problem of drug abuse in Russia and the growing number of hidden users of narcotic drugs (ND) require the list of screening examinations for ND identification to be extended by including more economical approaches that reduce costs at the stages of collection, transportation, storage, and analytical examination of biological samples.
Objective. Development of a multiplex immunoassay method based on the PHOSPHAN technology for detecting the main groups of narcotic and psychotropic substances in pools of paper-dried urine samples, followed by an assessment of its potential for identifying drug addicts as part of an extended drug control program.
Materials and methods. Dry urine samples (n = 31) were prepared on paper test strips from liquid samples containing (n = 30) or non-containing (n = 1) cocaine, cannabinoids, amphetamines, opiates, benzodiazepines, barbiturates, methamphetamine, or methadone, according to toxicology screening (TS). The samples were studied as pools containing 1–40 fragments (0.45×0.45 cm) of test strips. The luminescent signal was recorded on a microplate immunochip using an IFI-05 photoluminescence pulsed indicator. The ND presence in the samples was assessed by the inhibition rate of antibody binding in the related microplate test zone (B/B_0 ratio). Statistical processing of the results was carried out using the standard Microsoft Office package.
Results. The inclusion of dry urine samples in the pools (up to 10), where only one contained the target ND, had no significant effect on the capability of the method to detect NDs with sensitivity levels that meet the TS requirements. The following substances were detected: cocaine (2 samples), cannabinoids (11 samples), amphetamines (6 samples), opiates (9 samples), benzodiazepines (7 samples), barbiturates (10 samples), methamphetamine (7 samples), and methadone (6 samples), including samples with high concentrations of opiates and amphetamines.
Conclusions. A method of multiplex phosphorescence microplate immunoassay has been developed for the detection of eight main groups of NDs and psychotropic substances in pools of paper-dried urine samples (dried urine spot, DUS). The detection limits of the studied NDs in extracts from DUS test-strips were 2–8 ng/mL, which is significantly lower than the detection limits recommended for screening examination. The proposed approach can form the basis of a new screening methodology that includes collection of urine samples, their application onto filter paper test-strips, and transportation to a laboratory for the examination of individuals at industrial facilities of critical importance. The use of the developed multiplex phosphorescence immunoassay and pooled urine samples will significantly reduce the test cost (by more 10-fold) compared to conventional immunochromatographic assays.

Keywords: narcotic drugs; screening; time-resolved phosphorescence immunoassay; microplate immunochips; dry urine samples; pooling; toxicology screening

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ПЕРСПЕКТИВЫ ИНТЕГРАЦИИ МУЛЬТИПЛЕКСНОГО ФОСФОРЕСЦЕНТНОГО ИММУНОАНАЛИЗА ПУЛИРОВАННЫХ СУХИХ ОБРАЗЦОВ МОЧИ В СКРИНИНГОВОЕ ОБСЛЕДОВАНИЕ ПРИ ДИСПАНСЕРНОМ НАРКОКОНТРОЛЕ

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Введение. С учетом сложной наркоситуации в России, роста числа скрытых потребителей наркотических средств (НС) представляется целесообразным расширение рамок скрининговых обследований для выявления НС с использованием новых методических подходов, позволяющих сократить стоимость тестирования за счет снижения затрат на этапах сбора, транспортировки, хранения и аналитического исследования биологических образцов.

Цель. Разработка на основе технологии ФОСФАН метода мультиплексного иммуноанализа для обнаружения основных групп наркотических, психотропных средств в пулах образцов мочи, высушенных на бумаге, с последующей оценкой перспектив его применения для выявления наркозависимых лиц в рамках расширенного диспансерного наркоконтроля.

Материалы и методы. Сухие образцы мочи (n = 31) приготовлены на бумажных тест-полосках из жидких образцов, содержащих (n = 30) или не содержащих (n = 1) кокаин, каннабиноиды, амфетамин, опиаты, бензодиазепины, барбитураты, метамфетамин или метадон по данным химико-токсикологического исследования (ХТИ). Образцы исследованы в виде пулов, содержащих 1–40 фрагментов (0,45×0,45 см) тест-полосок. Люминесцентный сигнал регистрировали на микропланшетном иммуночипе с помощью индикатора фотолюминесценции импульсного ИФИ-05. Наличие НС в образцах оценивали по степени ингибирования связывания антител в соответствующей тестовой зоне иммуночипа (отношение B/B_0). Статистическую обработку результатов проводили с помощью стандартных программ Microsoft Office.

Результаты. Включение в состав пулов до 10 сухих образцов мочи, только один из которых содержит искомое НС, не оказало значимого влияния на способность разрабатываемого метода выявлять НС с показателями чувствительности, соответствующими требованиям ХТИ. В исследованных пулах обнаружены: кокаин — в 2 образцах, каннабиноиды — в 11 пробах, амфетамин — в 6, опиаты — в 9, бензодиазепины — в 7, барбитураты — в 10, метамфетамин — в 7, метадон — в 6 пробах, в том числе на фоне высоких концентраций опиатов и амфетаминов.

Выводы. Разработан метод мультиплексного фосфоресцентного микропланшетного иммуноанализа для выявления восьми основных групп наркотических, психотропных средств в пулах из высушенных на бумаге образцов мочи. Пределы детекции исследуемых НС в экстрактах из высушенных на тест-полосках образцов мочи составили 2–8 нг/мл, что существенно ниже рекомендованных для скринингового обследования пределов детекции. Предложенный подход может составить основу новой методологии скрининга, включающей отбор проб мочи, нанесение их на бумажные тест-бланки и транспортировку в лабораторию для проведения обследования групп лиц на критически важных объектах. Использование мультиплексного фосфоресцентного иммуноанализа и пулированных образцов мочи позволит кардинально (более чем в 10 раз) снизить стоимость тестирования по сравнению с традиционными технологиями иммунохроматографического анализа.

Ключевые слова: наркотические средства; скрининг; фосфоресцентный иммуноанализ с временным разрешением; микропланшетные иммуночипы; сухие образцы мочи; пулирование; химико-токсикологические исследования

Для цитирования: Бекман Н.И., Помелова В.Г., Осин Н.С. Перспективы интеграции мультиплексного фосфоресцентного иммуноанализа пулированных сухих образцов мочи в скрининговое обследование при диспансерном наркоконтроле. *Медицина экстремальных ситуаций*. 2025;27(3):400–409. https://doi.org/10.47183/mes.2025-261

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование проведено в соответствии с Хельсинкской декларацией «Этические принципы медицинских исследований с участием человека в качестве испытуемого» (2013 г.). Согласие на участие в исследовании не требовалось, поскольку в работе использованы деидентифицированные образцы мочи от анонимных пациентов.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Illicit use of narcotic drugs (ND) or their chemical analogues continues to be a global problem affecting approximately 292 million people (more than 5% of the world's adult population)[1]. In Russia, approximately 2% of the population aged 15–64 use narcotic drugs[2]. This is due not only to the large-scale import of opium ND from abroad, but also to the appearance of new groups of narcotic and psychotropic substances (such as synthetic cannabinoids and spice drugs) and the continued involvement of young people in drug consumption [1]. The current drug situation in Russia is associated with an increasing number of hidden drug users. This group includes people aged 30–40 with a high level of education and social status, who intentionally consume "lighter" drugs. This contributes to the development of drug addiction with delayed acute periods, escaping the attention of law enforcement agencies or medical professionals [2].

Currently, medical examinations for potentially dangerous substances are conducted in cases specified by Russian legislation as part of a two-stage procedure for toxicology screening[3]. However, the worsening drug situation and the growing number of hidden drug users requires stricter screening examinations for drug detection through the organization of dispensary drug control. In addition, even a single drug use can affect the person's ability to assess their surroundings and take appropriate actions in cases of emergency. Identification of drug addicts is particularly important considering the number of critically important and potentially dangerous infrastructure facilities in Russia, being directly related to ensuring the national security [3].

The organization of extended dispensary drug control requires significant financial expenditures. Thus, the cost of the most popular and affordable immunochromatographic test strips for detecting 6–8 main ND groups is at least 300 rubles, which practically excludes the possibility of introducing drug control at industrial

[1] United Nations Office on Drugs and Crime (UNODC). World Drug Report; 2024.
[2] Roberts L. Drug addiction in Russia: statistics, therapy, prevention. Modern-info.com. 2025.
[3] Federal Law 61-FZ On Medicine Circulation. Moscow.: Federation Council; 12.04.2010.

enterprises through budget funding. The cost of testing one sample for 8–13 main ND groups should be significantly lower for screening purposes. New methodological approaches that have emerged in recent years, including the pooling of test samples, the use of clinical samples dried on special paper, the centralization of laboratory tests, and the introduction of multiplex assay technologies, could significantly (by more than 10–20 times) reduce the test cost by optimizing costs at the stages of collection, transportation, storage, and sample assay [4–10].

The pooling technique, i.e., the combination of samples from different people is a promising approach to dispensary drug control [4–10]. An obvious advantage of group testing consists in the reduction of test costs in proportion to the sample number in the pool, the total number of tests, and the minimization of dosing errors [7]. The effectiveness of this approach was demonstrated during the COVID-19 pandemic, when the number of required examinations increased progressively [8–10]. However, there are still doubts about the possibility of widespread use of group testing in laboratory practice due to the increased risk of missing infected patients and the possibility of sample confusion during additional manipulations associated with sample pooling procedures [7].

Taking the above into account, the following criteria [11] were proposed to assess the applicability of the pooling technique for detecting the analyte in question:

1) the analyte concentration in the test patients should be at least 10 times higher than in healthy individuals;

2) the sample dilution should not significantly reduce clinical sensitivity;

3) the prevalence of the test pathology should be low, within 1–10%;

4) the absence of requirements for test speed;

5) the need for resource rationing in order to achieve maximum efficiency in the measures taken based on the test results.

In our opinion, the methodology for detecting ND in pooled samples meets almost all of the above-mentioned criteria, which justifies its further development in combination with the use of highly sensitive multiplex tests.

Laboratory tests centralization also significantly reduces costs by collecting samples of biological material (urine, blood, and other body fluids) as a spot dried on filter paper, which are then transported to a central laboratory for assay [12, 13]. Postal delivery of test forms does not require a cold chain and can be used to accumulate samples in a single (or regional) diagnostic examination center, similarly to the procedures for screening hereditary metabolic diseases in newborns. The reliability of testing for biologically active compounds, including ND, in paper-dried samples has served as the basis for the introduction of this technology into the global system of the World Anti-Doping Agency (WADA)[4] doping control of athletes.

Highly sensitive and cost-effective tools of detecting ND are required for the assay of pooled dry urine (or blood) samples. Conventional immunochromatographic assays are not suitable for this approach due to their low sensitivity and low accuracy. Gas chromatography–mass spectrometry shows higher sensitivity [14]; however, its potential for screening is limited by its high test cost and complexity.

A highly cost-effective solution is the development of a domestic technological platform for multiplex immunoassay based on microplate immunochips with time-resolved luminescent signal detection (PHOSPHAN™ technology) [15, 16]. Immunochips made using this technology are microarrays (microzones) at the bottom of 96-well microplates, each of which is designed to detect a specific type of neurotransmitter. The consumption of key reagents for creating such tests is reduced by many times compared to conventional immunochromatographic assays, while the microplate format of immunochips allows for parallel high-performance screening of multiple samples.

In this study, we aim to develop a multiplex immunoassay method based on the PHOSPHAN™ technology for detecting the main groups of narcotic and psychotropic substances in pools of paper-dried urine samples, followed by an assessment of its potential for identifying drug-addicted persons as part of extended drug control programs.

MATERIALS AND METHODS

The following immunobiologicals were used to create a test for detecting NDs: mouse monoclonal antibodies (MAb) to morphine (MOR), benzoylecgonine (BZE), amphetamine (AMP), methamphetamine (mAMP), methadone (MTD), benzodiazepine (BZD), barbiturates (BAR) and Δ9-tetra-hydrocannabinol (THC) (CALBIOREAGENTS Inc., USA), biotin-labeled according to the standard procedure (SIGMA, USA); conjugates of MOR, BZE, AMP, mAMP, MTD, BZD, BAR and THC with bovine serum albumin (CALBIOREAGENTS, USA).

A conjugate of streptavidin with Pt-coproporphyrin (Immunoscreen, Russia) was used as a detection reagent.

Dry samples for the study were prepared from 50 liquid human urine samples containing various narcotic and non-narcotic compounds in various combinations and concentrations (the samples were provided by the Sechenov First Moscow State Medical University). The liquid samples were certified based on the results of toxicology screening (TS)[5], and were also previously characterized in the NARK-PHOSPHAN multiplex test [15].

[4] WADA. Technical Document Dried Blood Spots (DBS) for Doping Control. 2023.

[5] Methodical Guidelines “Rules for conducting chemical and toxicological tests for narcotic drugs, psychotropic substances, and other toxic substances (their metabolites) in the human body during medical examinations and medical assessments of certain citizens categories”. Moscow.: Ministry of Health of the Russian Federation; 2015.

To prepare dry samples, test strips (blanks) of filter paper (WHATMAN 903, USA) 0.45 cm wide and at least 3–4 cm long were used; the strips were soaked in urine by immersing them in a container with a liquid sample for 1 min, followed by air drying. The blanks were stored at a temperature of +4°C with a desiccant in a foil bag. The assay was performed using 0.45×0.45 cm fragments of a paper test strip containing dry material equivalent to 8 ± 0.4 μL of liquid urine. WHATMAN 903 paper ensured a standard sample volume per unit area and good preservation of the biomaterial.

Dry urine samples (31 samples in total) were prepared from 50 liquid samples as follows: 30 dry samples were prepared from 30 liquid samples contained ND in different combinations and concentrations; one sample (a negative dry sample) was prepared from a mixture of 20 liquid urine samples that contained various common medications but no drug compounds [15].

To study the stability of ND in dry samples during storage, the prepared samples were kept at temperatures of 25 ± 5°C, 4–8°C, or –20°C for 14 days, 6 months, or 12 months, respectively. The effect of biosample storage temperature was considered insignificant provided that the difference in the ND level before and after sample storage was less than 15%.

A modified version of the NARK-PHOSPHAN test system developed by us earlier [15] was used to analyze dry urine samples. Immunochips were eight test microarrays printed on the well bottom of a 96-well polystyrene plate (NUNC, Denmark) to detect the studied ND (BZE (cocaine); THC (cannabinoids); AMP (amphetamine); MOR (opiates); BZD (benzodiazepines); BAR (barbiturates); mAMP (methamphetamine); MTD (methadone), and one control zone C. The test zones sorbed the related ND-protein (serum albumin) conjugates, and the control zone sorbed the biotin-protein (serum albumin) conjugate (Fig. 1). The immunochips were printed using the technique of non-contact printing, which reduced the microarray variability and reduced the sorbable immunoreagent volume to 2.5 nL compared to the previous test system [15].

The created immunoassay chip allows eight ND (MOR, BZE, THC, MTD, BZD, BAR, AMP and mAMP) to be detected in one plate's well. This assay is based on a competitive reaction between test sample ND and ND conjugates in the immunochip test zones for binding with biotinylated ND antibodies. In the absence of ND in the sample, specific biotin-labeled MAbs bind to the related microarrays in the plate wells, which are then detected using streptavidin labeled with the long-lived luminescent marker Pt-coproporphyrin. In the presence of ND in the sample, a portion of the specific biotinylated antibodies binds to ND and is removed during washing of the plate wells. The higher the ND concentration in the sample, the lower the residual signal level in the test microarray.

The layout of immunoassay drug test using paper test strips and pooled samples is presented in Fig. 2.

Square fragments measuring 0.45×0.45 cm were cut off test strips impregnated with urine samples along the perforation line and placed in the wells of an auxiliary non-absorbing flat-bottomed plate. An extracting solution (the solution volume varied 25–500 μL) containing a mixture of mouse biotinylated MAbs was added to each well, and the samples were incubated for 30 min. Then, 25 μL of the resulting reaction mixture was transferred into the plate wells with printed immunochips and incubated for 1 h. After washing, 25 μL of streptavidin-Pt-coproporphyrin conjugate was added to the wells and incubated for 15 min. The MAb and conjugate dilutions were prepared in a buffer (pH 7.75) solution containing 12.1 mg/mL of tris-(hydroxymethyl)-aminomethane, 0.1 mL/L of Tween 20, 0.5 mg/mL of BSA (bovine serum albumin), 8.7 mg/mL of sodium chloride, 0.5 mg/mL of sodium azide (all reagents from SIGMA, USA). All incubations were carried out upon shaking at 700 rpm at room temperature. The plate was then washed and dried.

The ND presence in the sample was assessed by the inhibition rate of antibody binding in the related test zone using the B/B_0 ratio (B is the level of the phosphorescent signal at a given ND concentration, and B_0 is the level

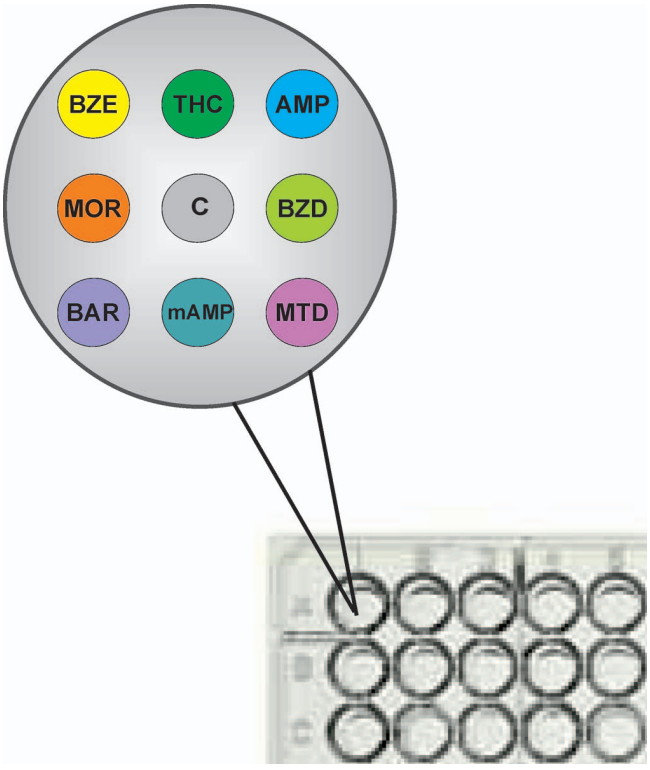

Figure prepared by the authors

Fig. 1. Layout of immunochip microarrays at the well bottom of 96-well microplates: BZE (cocaine); THC (cannabinoids); AMP (amphetamine); MOR (opiates); BZD (benzodiazepines); BAR (barbiturates); mAMP (methamphetamine); MTD (methadone); C (control)

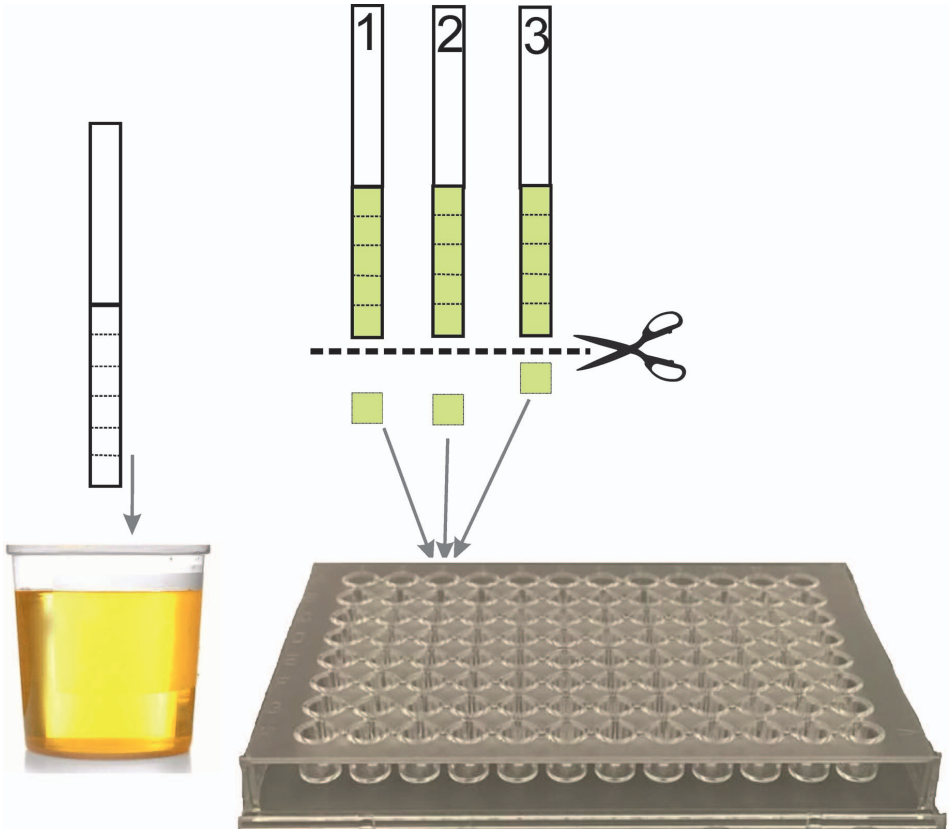

Figure was prepared by the authors

Fig. 2. Layout of urine drug test using paper test strips and the pooling procedure

of the phosphorescent signal at zero ND concentration), which characterizes the inhibition rate of the phosphorescence signal in the related test zone of the immunoassay. The performance of the procedure was monitored by the signal level detected in the control zone of the immunochip, where the streptavidin conjugate binds to the Pt-coproporphyrin luminescent label.

The phosphorescent signal was recorded using an IFI-05 photoluminescence pulse indicator (Immunoscreen, Russia) by scanning the bottom of the plate well with light pulses with an excitation wavelength of 365 nm in stroboscopic mode with a pulse repetition frequency of 10 kHz and the selection of the long-lived luminescence signal of Pt-coproporphyrin with a maximum of 645 nm and a decay time constant of 40 μs. The results were processed and presented using the indicator software. For each test sample and detected ND, the ratio (B/B_0) of the phosphorescence signal intensities recorded in the related immunochip test zone in the wells with the test sample (B) and without the addition of the B_0 sample was automatically calculated.

Measurements were carried out in triplicate with calculation of the mean value (M) of the measurement results and the standard error of mean (SE). For statistical processing of the results, the standard Microsoft Office Professional Plus Excel 2013 (version 15.0.4727.1000, USA) software was used.

RESULTS

When developing a multiplex immunoassay technology using pooled dried samples, the first step was to evaluate the detection sensitivity of each target protein. To that end, test strips were analyzed using urine samples with known levels of the target proteins. To prepare the model extracts, test strip fragments were placed in an extracting solution of varying volumes, ensuring that the target proteins concentration in the resulting reaction mixtures ranged 5–300 ng/mL. Based on the inhibition curves of the recorded signal for each of the eight ND (Fig. 3), the probable detection limits of the PHOSPHAN assay (as the ND levels corresponding to the recorded signal intensity value in the assay of a sample not containing this ND, minus two standard deviations) were calculated, which turned out to be 3–150 times lower for different NDs than the values recommended for toxicology screening tests (see Table).

Prior to examination of pooled samples, it was necessary to take into account that an increase in the number of simultaneously analyzed test strip fragments leads to an increase in the volume of the extracting solution required to fully saturate all the studied ND fragments, followed by the selection of 25 μL of the resulting reaction mixture for assay using immunochips. Our studies have shown that 50 μL of extracting solution

is sufficient for analyzing a single fragment; for pools of 5, 10, 20, and 40 samples, the minimum volume of solution was 100, 160, 240, and 400 μL, respectively. Therefore, the urine sample dilution increased by a factor of 2, 3, 5, and 8, respectively, compared to the single sample assay.

Figure 4 shows an immunochip using results of testing of four dried human urine samples containing eight NDs in different combinations and concentrations; these samples were analyzed individually and as part of pools of 5, 10, 20, and 40 samples. Each pool contained one positive sample and a different number of strips of negative dried sample.

For all the samples studied, the B/B_0 ratios in the test zones for the specific binding of drug compounds presented in the sample increased linearly from extremely low values to 75% along with an increase in the pooling frequency. The B/B_0 ratios calculated in the test zones for the detection of drug compounds absent in the sample varied 70–110% (Fig. 4).

The conducted experiments (Fig. 4) allowed us to conclude that, in terms of analytical and economic parameters, pools of no more than 10 samples should be used. In this case, the B/B_0 ratio recorded in the specific test zones of the immunochip in the presence of ND did not exceed 40% for all the samples studied. The ranges of the B/B_0 parameter values in the test zones in the presence of ND did not overlap with the ranges of values recorded in the absence of ND in the sample. Based on the results obtained, the B/B_0 value of 50% was selected as the threshold level for detecting ND.

Using the selected assay procedure based on 10-fold pooling (1 sample with ND + 9 samples without ND), we examined 30 dry samples containing initial liquid urine samples in various combinations of ND with the concentration ranges of 168–230 ng/mL BZE, 81–206 ng/mL THC, 405–1250 ng/mL AMP, 35–2500 ng/mL MOR, 251–800 ng/mL BZD, 75–350 ng/mL BAR, 115–471 ng/mL mAMP and 84–375 ng/mL MTD. The results are presented in Fig. 5, where the analysis data for samples containing and not containing the corresponding ND are shown separately for each test zone.

According to the data obtained (Fig. 5), the following substances were detected in the studied pools: cocaine (2 samples), cannabinoids (11 samples),

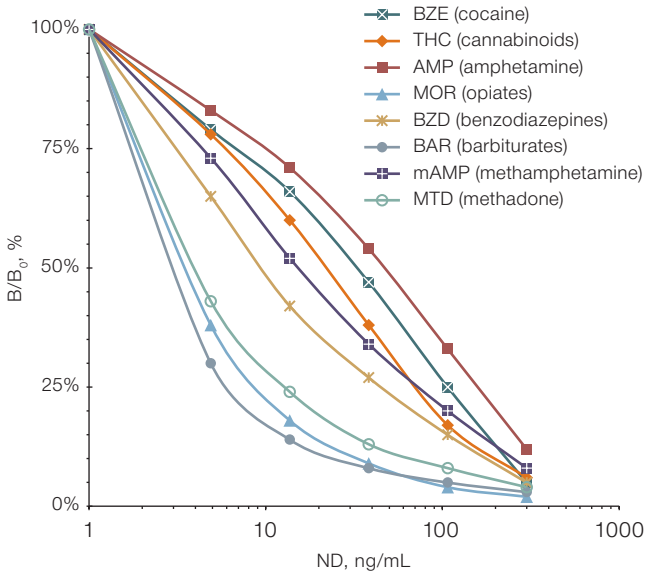

Figure prepared by the authors

Fig. 3. Inhibition curves of luminescent signals when eight NDs are detected in model extracts from urine samples dried on test strips: the data are presented in the form of the mean value (*M*) of the measurement results (n = 3); the standard error of the mean (*SE*) did not exceed 15%

Table. ND detection sensitivity in model extracts from urine samples dried on test strips using the PHOSPHAN technique

Narcotic drugs, psychotropic substances	Analyte	Proposed drug detection limits for toxicology screen, ng/mL		Estimated drug detection limit according to PHOSPHAN, ng/mL
		Screening	Confirmation	
Opiates	MOR	300	10	2
Cocaine	BZE	25	50	8
Cannabinoids	THC	15	15	6
Amphetamine	AMP	25	20	8
Methamphetamine	MAMP	25	20	6
Methadone	MTD	25	50	2
Benzodiazepines	BZD	20	50	4
Barbiturates	BAR	50	100	2

Table prepared by the authors based on their own data

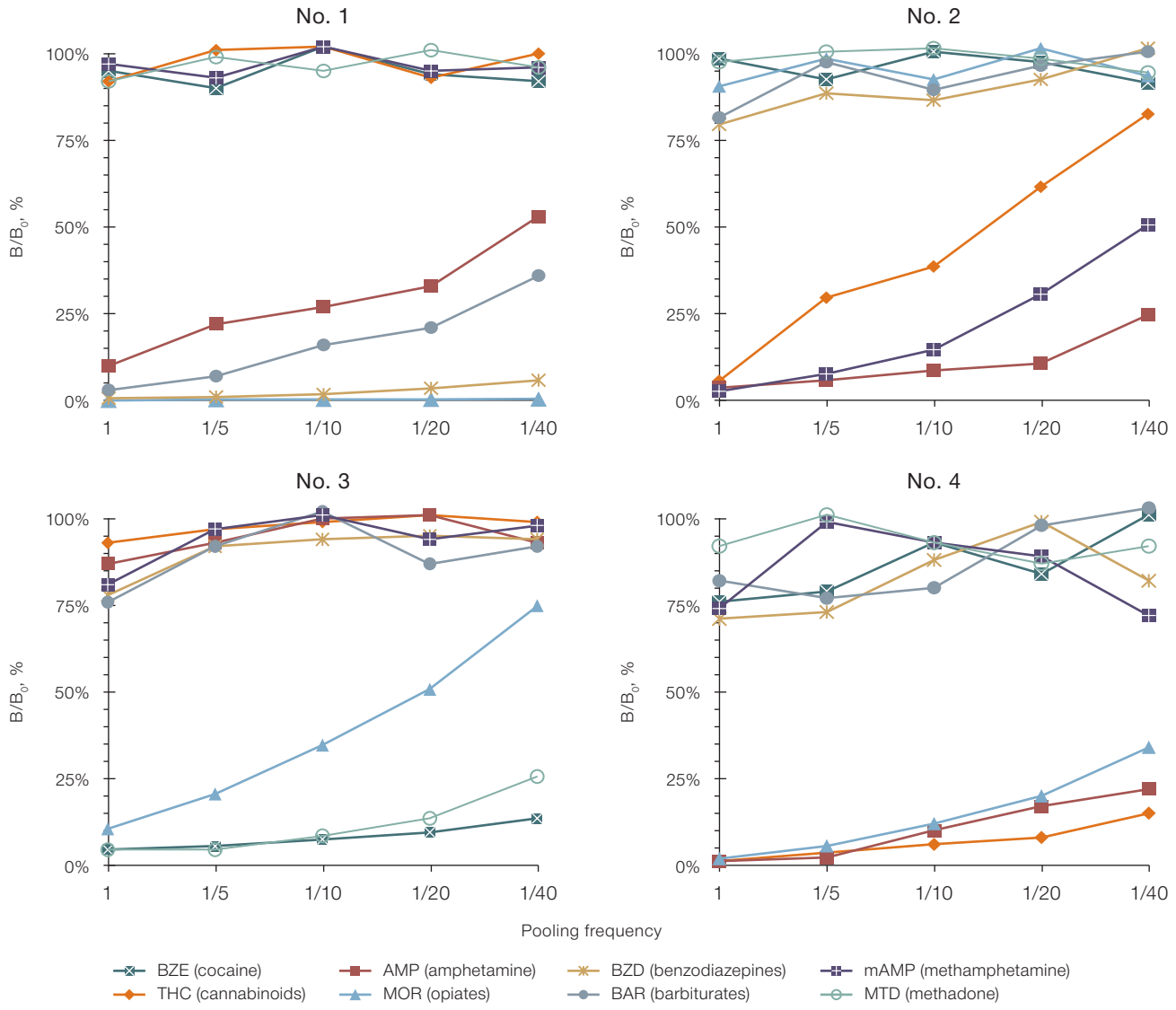

Figure prepared by the authors

Fig. 4. Phosphorescence signal inhibition rate when detecting eight NDs in dry urine samples (No. 1–4) using the PHOSPHAN technique, based on pooling frequency: ND baseline level in liquid urine samples (before drying on a paper test strip): sample No. 1 — 405 ng/mL AMP, 2500 ng/mL MOR, 506 ng/mL BZD and 75 ng/mL BAR; sample No. 2 — 115 ng/mL THC, 1250 ng/mL AMP and 471 ng/mL mAMP; sample No. 3 — 230 ng/mL BZE, 36 ng/mL MOR and 375 ng/mL MTD; sample No. 4 — 206 ng/mL THC, 1250 ng/mL AMP and 2500 ng/mL MOR; the data are presented in the form of the mean value (*M*) of the measurement results (n = 3); the standard error of the mean (*SE*) did not exceed 15%

amphetamines (6 samples), opiates (9 samples), benzodiazepines (7 samples), barbiturates (10 samples), methamphetamine (7 samples), and methadone (6 samples). Some samples contained both cocaine and methadone, benzodiazepines and barbiturates, as well as other ND mixtures in the presence of very high concentrations of opiates (> 2500 ng/mL) and amphetamines (> 1250 ng/mL).

For all positive dry samples containing ND, the B/B_0 values in the related specific test zones were significantly lower than 50%. For the remaining samples containing other ND, the values were significantly higher than 50% (Fig. 5). Therefore, the developed multiplex phosphorescence immunoassay technique correctly detected the target ND in pools of 10 dry urine samples without any false-positive results with other ND.

The immunoassay specificity was also evaluated based on the study results of three pools composed of 10 test strip fragments from a negative urine sample. This sample was prepared from a mixture of liquid samples from patients whose urine contained various non-narcotic medications, including those that can cause false-positive reactions in the immunoassay (e.g., carbamazepine, amitriptyline, dextromethorphan, verapamil, etc.) [17]. During testing, the target NDs were not detected in any of the pools studied, which confirmed the specificity of the developed multiplex phosphorescence immunoassay technique.

DISCUSSION

When developing a technological platform for multiplex immunoassay using pooled dry urine samples, we aimed to ensure its sensitivity and specificity comparable to those of highly sensitive reference methods of gas chromatography–mass spectrometry [12–14]. The development was based on the technology of multiplex phosphorescent immunoassay on microplate immunochips, which had previously demonstrated the possibility of achieving low detection limits for ND in liquid urine samples (sensitivity was 1 ng/mL for morphine and methadone, 0.5 ng/mL — for barbiturates, 2 ng/mL — for benzoylecgonine, methamphetamine, cannabinoids and benzodiazepines, 8 ng/mL — for amphetamine) in the absence of significant cross-reactions [15].

The study objective was to modify the PHOSPHAN technology for the assay of pooled dry samples. It is known that the assay of such samples is accompanied by an inevitable decrease in sensitivity due to incomplete desorption and additional dilution during the extraction of analytes from the paper. To address this issue, we employed a series of technological approaches to enhance the sensitivity of the modified PHOSPHAN tests while maintaining their specificity.

The immunochip formation was carried out using a non-contact printing method with a microarray volume of 2.5 nL (instead of 20–25 nL in the previously used contact printing method) [15]. This reduction in the amount of conjugated ND antigen in the test microzone of the immunochip resulted in a 2–3-fold decrease in the ND detection limit in competitive immunoassay.

The method includes a step of ND pre-extraction from paper test strips (examined as fragments of 4.5×4.5 mm in size) in the presence of specific biotinylated antibodies. The pre-interaction of the ND sample with antibodies in the absence of competition with the BSA conjugate for the ND provided a greater inhibition rate and, accordingly, an additional reduction in the detection threshold.

The method of preparing dry samples by immersing the test strip in a container with a human urine sample ensures soaking of a sufficiently large area, which allows for up to five repeated measurements of the sample (Fig. 2), including for confirmation testing in the event of ND detection in the pool examined. The dimensions of the analyzed test strip fragments (4.5 x 4.5 mm) allow for the pooling of up to 10 samples in a single microplate well.

The proposed method for ND assay from a pool of dry urine samples can form the basis for a new screening technique that involves collecting urine samples, their application onto paper test forms, and transportation to a laboratory, including by mail, for ND testing.

It is important to note that there are no special requirements for the “cold chain” when shipping dry samples, as the ND level in paper-dried urine samples remains relatively stable. According to our research, the levels of eight analytes under study decreased by no more than 15% during storage for five days at room temperature,

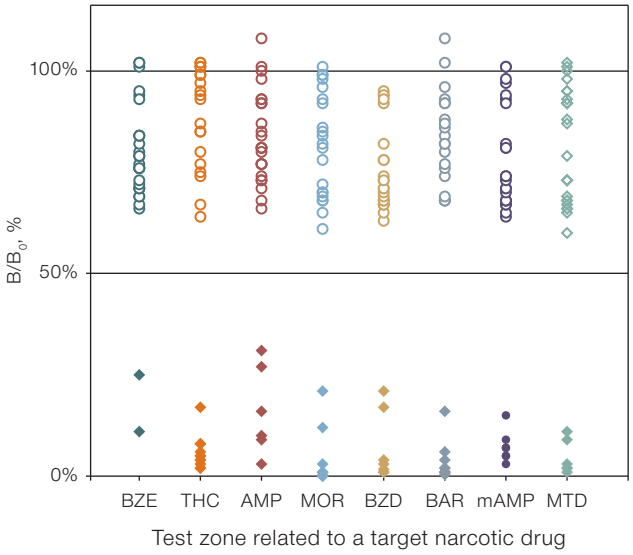

Figure prepared by the authors

Fig. 5. Results of detecting eight NDs in 30 dry urine samples examined as pools of 10 samples using the multiplex PHOSPHAN technique on microplate immunochips: BZE (cocaine); THC (cannabinoids); AMP (amphetamine); MOR (opiates); BZD (benzodiazepines); BAR (barbiturates); mAMP (methamphetamine); MTD (methadone); rhomb — samples contained ND, circle — samples non-contained ND, corresponding to this test area; the ordinate axis shows the average values for three repeated measurements

one month at 4°C, and up to six months in frozen form. This is consistent with the findings of other researchers regarding a wide range of analytes detected in paper-dried clinical urine and blood samples [14].

In the laboratory, the collected samples can be combined into pools of 10 samples and subjected to a preliminary assay, where each pool is tested in a single well of a microplate immunoassay. In cases where the pool produces a positive result for one of the target ND, a confirmation test is performed, which involves testing each of the 10 samples in the pool and determining the exact concentration of the ND in the sample using the corresponding calibration curves provided in the biochip analyzer software.

On the basis of the results obtained, we set the threshold level for recognizing a test result as positive to B/B_0 = 50%. This criterion should be defined more precisely by conducting research using a wider sampling. It may be necessary to select different evaluation criteria for each analyzed ND in order to reduce the likelihood of obtaining false-positive or false-negative results.

Due to its high sensitivity, the developed method of multiplex phosphorescence immunoassay allowed the detection of eight main NDs in a pool of 10 samples. The specificity of the method was confirmed by the correctness of ND identification in all the studied samples, including those containing other NDs, and the absence of non-specific reactions when analyzing a

pool of negative urine samples containing various non-narcotic medications.

The proposed technological platform has no fundamental limitations for extending the panel of NDs detected in a single immunoassay. Our preliminary experiments have shown that it is possible to additionally include synthetic cannabinoids (K2) and cathinones (MDPV), phencyclidine, fentanyl, and ecstasy (MDMA) in the detected ND list, since their sensitivity and specificity meet the requirements for test development using the sample pooling technique. Thus, the number of simultaneously detectable substances can be extended to at least 13, covering all groups of substances that must be monitored during chemical and toxicological examinations of drug-dependent individuals.

CONCLUSIONS

1. A multiplex phosphorescence microplate immunoassay has been developed for detecting eight main groups of narcotic and psychotropic substances in pools of paper-dried urine samples.

2. The detection limits of the target ND in extracts from urine samples dried on test strips ranged 2–8 ng/mL, which is significantly lower than the detection limits recommended for screening examinations.

3. The proposed approach to multiplex immunoassay of NDs in pools of dry urine samples can form the basis of a new screening technique that includes urine sampling, application to paper test forms, and transportation to a laboratory for ND testing.

4. According to our estimates, the use of multiplex phosphorescence immunoassay and pooled urine samples will significantly (by more than 10 times) reduce the cost of testing compared to conventional immunochromatographic assay technologies. A centralized laboratory equipped with a high-performance domestic photoluminescence indicator of the IFI-05 series, can process at least 10,000 samples per work shift, divided into pools of 10 samples. During mass examinations of groups of people at critically important industrial facilities, the cost of one examination for 8–13 NDs will not exceed 20–30 rubles, or 2–3 rubles per one type of ND detected.

5. The work conducted by specialists of the State Scientific Research Institute of Biological Engineering and the Immunoscreen company is aimed at further development of highly cost-effective domestic technologies for ND screening using pooled urine samples and conducting pilot surveys, thus contributing to organization of improved dispensary drug control in the entitled territories of the Federal Medical and Biological Agency (FMBA) of Russia.

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CROHN'S DISEASE COMPLICATING A RARE PRIMARY IMMUNODEFICIENCY IN A PEDIATRIC PATIENT: CHALLENGES IN MEDICAL AND SURGICAL DECISION-MAKING

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Introduction. Primary immunodeficiency disorders (PID) are a group of congenital diseases caused by genetic defects that lead to diverse phenotypic manifestations. Classic inflammatory bowel diseases (IBD) are typically multifactorial pathologies, combining genetic predisposition, gut microbiota alterations, and adverse environmental influences. Very early-onset IBD (VEO-IBD), defined as a disease presenting before six years of age, accounts for 3–15% of all pediatric IBD. This subgroup is particularly characterized by monogenic etiology, associated with gastrointestinal phenotype PID and with causative mutations in specific genes.
Case report. We present a clinical case of monogenic Crohn's disease in a child with X-linked lymphoproliferative syndrome type 2 (XLP-2), treated using a multi-stage team approach. To achieve sustained remission, in addition to selecting conservative therapy, repeated surgical interventions and repeated hematopoietic stem cell transplantations were required. An individualized approach and treatment strategy planning enabled a positive treatment outcome.
Conclusions. In young children with the presence of atypical IBD and refractoriness to standard therapy, it is crucial to differentiate monogenic forms of IBD. Such patients require close monitoring, dynamic follow-up, and a multidisciplinary approach involving collaboration between gastroenterologists, immunologists, and surgeons.

Keywords: Crohn's disease; primary immunodeficiency; X-linked lymphoproliferative syndrome; children

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БОЛЕЗНЬ КРОНА У РЕБЕНКА С РЕДКИМ ПЕРВИЧНЫМ ИММУНОДЕФИЦИТНЫМ СОСТОЯНИЕМ: ОСОБЕННОСТИ КОНСЕРВАТИВНОЙ И ХИРУРГИЧЕСКОЙ ТАКТИКИ

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Введение. Первичные иммунодефицитные состояния (ПИДС) — это группа врожденных заболеваний, вызванных генетическими дефектами и обусловливающих разнообразные фенотипические проявления. Классические воспалительные заболевания кишечника (ВЗК) в большинстве случаев являются многофакторными патологиями, когда сочетаются генетическая предрасположенность, изменения кишечной микрофлоры и неблагоприятное влияние окружающей среды. Очень раннее начало ВЗК, с дебютом болезни в возрасте до 6 лет, составляет 3–15% всех детских воспалительных заболеваний кишечника. Именно для этой группы характерна моногенная этиология, протекающая в рамках ПИДС с гастроинтестинальным фенотипом и связанная с мутацией в конкретном гене.
Описание клинического случая. Представлен клинический случай моногенной болезни Крона у ребенка с Х-сцепленным лимфопролиферативным синдромом 2-го типа с демонстрацией многоэтапного командного подхода. Для достижения стойкой ремиссии, помимо подбора консервативной терапии, потребовалось проведение повторных хирургических вмешательств и повторных трансплантаций гемопоэтических стволовых клеток. Индивидуальный подход и планирование лечебной стратегии позволили достичь положительного результата лечения.
Выводы. Очень важно у детей раннего возраста с атипичным течением воспалительного заболевания кишечника и рефрактерностью к стандартной терапии дифференцировать моногенные формы ВЗК. Такие пациенты требуют пристального внимания, динамического наблюдения, мультидисциплинарного подхода с альянсом гастроэнтеролога, иммунолога и хирурга.

Ключевые слова: болезнь Крона; первичное иммунодефицитное состояние; Х-сцепленный лимфопролиферативный синдром; дети

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: от законных представителей пациента получено письменное информированное добровольное согласие на публикацию описания клинического случая, обезличенных медицинских данных и фотографий.

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INTRODUCTION

Primary immunodeficiency disorders (PID) are genetically determined diseases characterized by congenital defects in the body's defense mechanisms, leading to impaired immune responses with the development of recurrent infections, increased risk of malignancies, and autoimmune diseases [1]. The current classification of PID adopted by the International Union of Immunological Societies (IUIS) takes into account molecular genetic defects in various immunodeficiencies, identifying 10 main groups of PID [2].

X-linked lymphoproliferative syndrome (XLP) belongs to the group of PID with immune dysregulation; XLP is caused by mutations in the *SH2D1A*, *XIAP*, and *MAGT1* genes, characterized by an atypical response to Epstein–Barr virus infection and the development of hemophagocytosis, dysgammaglobulinemia, and malignant lymphoproliferation. The former two types (XLP1 and XLP2) are among the most studied types, which occur with a frequency of 1–3 per 1 million born boys, generating interest given the rarity of this disease. A significant difference between XLP2 and XLP1 is the development of hemorrhagic colitis, which clinically and morphologically resembles inflammatory bowel disease (IBD).

IBDs are classified as polygenic inherited disorders, with more than a hundred susceptibility loci that increase the risk of the disease having been identified. To date, over 300 , mutations in which lead to the development of PID genes, have been studied [3]. Among these, more than 75 genes whose mutations are associated with an IBD-like phenotype have been found [4]. In children with very early-onset IBD (VEO IBD) (before 6 years of age) and especially infantile IBD (with the disease onset before 2 years of age), differential diagnosis with PID is necessary [5]. IBD in the setting of a monogenic disease almost always follows a course similar to classical multifactorial IBD, differing in refractoriness to therapy. This may be the sole manifestation of PID at the time of presentation, delaying the verification of a final diagnosis [6]. The main treatment methods for PID include conservative therapy (symptomatic, antibacterial, and antifungal), immunoglobulin replacement therapy, treatment with genetically engineered biological drugs (GEBD) and targeted immunosuppressive agents, and in certain cases, hematopoietic stem cell transplantation (HSCT) [7].

However, to date, versatile approaches to treating such patients have been lacking. An important separate treatment option for patients with PID involving the colon is minimally invasive surgical interventions, such as preventive ileostomy before HSCT to reduce the risk of transplantation complications [8].

We present a clinical case of a pediatric patient with PID and Crohn's-like intestinal involvement, discussing the challenges of therapeutic and surgical treatment stages.

CASE REPORT

Patient B., male, 8 years old (born in 2016), was under observation at the gastroenterology department of the Federal Research and Clinical Center for Children and Adolescents of FMBA with the following diagnoses:

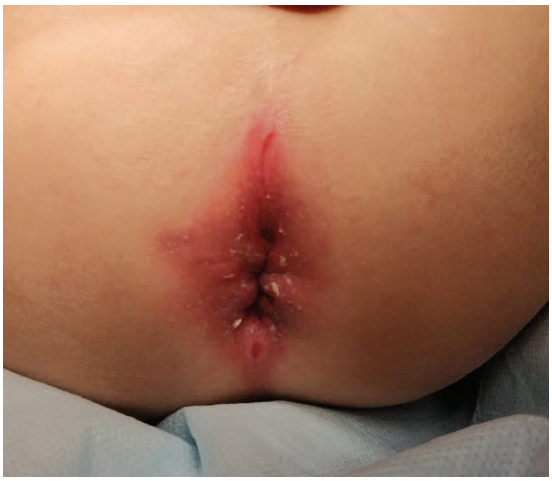
- Crohn's disease of the colon, moderate severity, minimal activity (PCDAI 10 points);
- Primary immunodeficiency, X-linked lymphoproliferative syndrome type 2 (mutation of the *BIRC4 (XIAP)* gene c.599G > A (p.Cys200Tyr) in hemizygous state;
- Autoimmune hemolytic anemia, drug-induced remission.

Life history

The child was born from the 2nd physiological pregnancy, 2nd term spontaneous delivery (1st child — girl, 10 years old — healthy). Birth length was 49 cm, and birth weight was 2860 g. The Apgar score was 8/8. The child was breastfed from the first day, continued breastfeeding until 1.5 years old. BCG (Calmette-Guerin bacillus) vaccinated at birth. The history of infections: frequent ARVI (at 1 month — ARVI with respiratory failure), pneumonia at 1 year. Routine vaccinations according to national schedule (medical exemption since 07.2017). Diaskintest (29.08.2017) was negative. No significant family history.

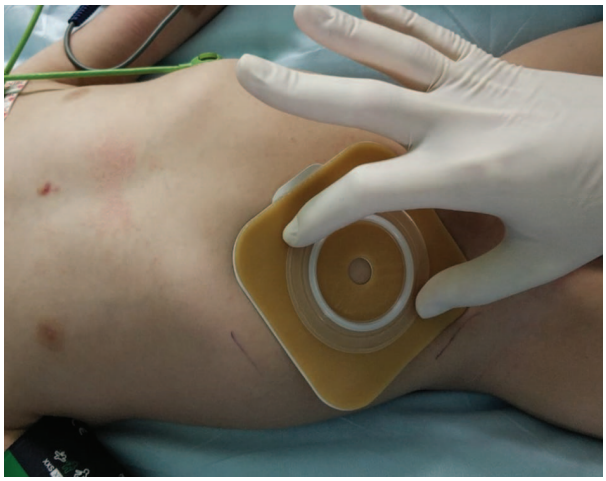
Medical history

From 3 weeks of age, the child had frequent loose stools with mucus, and from 9 months — blood streaks in stool. At 1 year old, the child developed pneumonia followed by several months of febrile episodes. In May 2017 (1 year 4 months), surgical treatment for acute



Clinical photo taken by the authors

Fig. 1. Perianal region appearance. Multiple anal fissures and perianal dermatitis were observed



Clinical photo taken by the authors

Fig. 2. Preoperative stoma site marking

paraproctitis (abscess incision and drainage) was performed.

Clinical deterioration in July 2017 led to hospitalization at the gastroenterology department of Morozov Children's City Clinical Hospital. On examination: fistulous opening with purulent discharge in the anal area, perianal dermatitis with maceration, anal fissure (Fig. 1).

Laboratory findings revealed: leukocytosis 22.3 × 10^3 cells/μL, decreased hemoglobin to 57 g/L, elevated C-reactive protein (CRP) to 64.9 mg/L, and immunogram abnormalities (leukocyte differentiation cluster — CD^{3+} 2000/μL, CD^{4+} 670/μL, CD^{8+} 1335/μL, CD^{19+} 339/μL, NK cells 55/μL). During hospitalization at the gastroenterology department in August 2017, an axillary lymphadenitis episode was noted, requiring incision and drainage of the abscess.

Colonoscopy identified ulcerative lesions of the colon, while histological examination revealed features of chronic left-sided ulcerative colitis with high activity. In 2017, gastroenterologists determined the diagnosis: Crohn's disease affecting the colon, with suspected primary immunodeficiency. Treatment was initiated with prednisone and 5-ASA (5-amino salicylic acid) drugs, supplemented by antimicrobial and antifungal therapy (amoxicillin + clavulanic acid, ciprofloxacin, cefoperazone + sulbactam, fluconazole). The child was referred for genetic testing (whole-exome genome sequencing).

In February 2018, molecular genetic testing confirmed a pathogenic variant in exon 2 of the *XIAP* gene: c.599G > A (p.Cys200Tyr) in hemizygous state. In March 2018, due to progressive deterioration marked by severe colitis syndrome, the child was admitted to the immunology department of Dmitry Rogachev National Research Center.

The patient was subscribed:

- TNF-α inhibitor therapy (adalimumab 40 mg SC weekly)
- Concurrent IL-6 receptor antagonist (tocilizumab) for hemophagocytic lymphohistiocytosis (HLH) prophylaxis

A medical consensus recommended diverting ileostomy prior to hematopoietic stem cell transplantation (HSCT) to reduce complications. Preoperative stoma site marking (Fig. 2) and laparoscopic-assisted double-barrel ileostomy (01.06.2018) was performed.

Laparoscopic examination of the abdominal cavity revealed the following findings: the right colon had normal wall thickness but was dilated to 4 cm in diameter; the sigmoid colon showed marked inflammatory changes over an 8–10 cm segment with thickened walls and creeping mesentery. The postoperative course was uneventful.

Subsequent treatment was conducted at the Dmitry Rogachev National Research Center. An allogeneic hematopoietic stem cell transplantation from a related haploidentical donor (mother) with TCRαβ/CD19 depletion was performed on 25.07.2018. On 15.08.2018, a complication occurred, involving ultra-early graft rejection.

Given the graft rejection, severe enteritis, and high ileostomy output (laboratory tests showed no infectious process with restored native leukopoiesis), therapy with recombinant monoclonal antibodies adalimumab 40 mg every 2 weeks was reinitiated.

A second allogeneic hematopoietic stem cell transplantation from a haploidentical donor (father) with TCRαβ/CD19-depleted graft processing was performed on 29.11.2018. Autoimmune hemolysis developed in early May 2019 (anemia with hemoglobin dropping to 73 g/L, reticulocytes 10%, direct Coombs test ++++). The child showed a progressive shift toward host chimerism and developed autoimmune hemolysis

refractory to IV immunoglobulin, prompting initiation of rituximab therapy at an initial dose of 375 mg/m^2 with positive effect.

In August 2019, the child was admitted to the Dmitry Rogachev National Research Center with severe pain and bowel evagination through the stoma. Conservative reduction attempts under tramadol analgesia and mask anesthesia were unsuccessful. Necrosis of the evaginated bowel was diagnosed, and emergency surgery was performed: bowel resection with creation of a double-barreled divided ileostomy. Postoperative wound dehiscence required secondary surgical wound debridement and stoma revision.

In September 2019, the child was admitted to the surgical department of the Russian Children's Clinical Hospital with peristomal complications (Fig. 3). Stoma therapy was performed with positive effect. Endoscopic examination of the excluded colon (Fig. 4) revealed erosive proctosigmoiditis and sigmoid colon stricture with lumen obliteration.

On 01.11.2019, at the Dmitry Rogachev National Research Center, graft rejection was confirmed based on bone marrow chimerism analysis (93.7% host cells). A discussion was held with the patient's parents regarding the need for repeat hematopoietic stem cell transplantation and associated risks. Given persistent erosive changes and stricture development in the excluded colon segments, the patient continued local therapy with Salofalk, adalimumab therapy at a reduced frequency of 40 mg weekly, initiated immunosuppressive therapy with azathioprine, and maintained complex antimicrobial therapy with intravenous immunoglobulin replacement. Repeat HSCT was declined due to autoimmune hemolytic anemia, granulocytic graft hypofunction, and severe colitis syndrome.

Considering the extremely high risk of worsening gastrointestinal damage from diversion colitis in the excluded intestinal segments, the decision was made to perform reconstructive surgery with resection of the strictured bowel segment, simultaneous ileostomy closure, and intestinal continuity restoration.

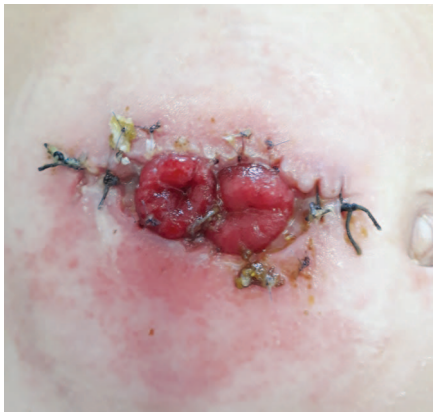
On 27.11.2019, at the Russian Children's Clinical Hospital (RCCH), the following elective procedures were performed: resection of the sigmoid colon stricture with colocolic anastomosis; ileostomy closure with ileocolonic anastomosis. Intraoperative examination revealed the entire colon to appear thickened, reduced in diameter (typical "excluded bowel" appearance), with markedly thickened mesentery and serosal surface nearly obscured by "creeping fat" (Fig. 5). A 4 cm segment of significant luminal narrowing was identified in the sigmoid colon. The efferent ileostomy limb was located 2 cm from the ileocecal valve, with the appendix embedded in adhesions between the afferent and efferent limbs. Resection of the ileocecal region was performed with end-to-end ileoascending anastomosis (Fig. 6).

Histopathological examination revealed:

- Macroscopic description: A segment of intestinal wall with adjacent adipose tissue measuring 4×3×1 cm. The mucosal surface showed no visible pathological changes. A separate segment from the ileocecal region including the appendix measuring 5.5×3.5×2 cm.
- Microscopic description and conclusion: The terminal ileum segment demonstrated preserved mucosa without inflammatory activity. Colonic wall fragments showed normal histological architecture.

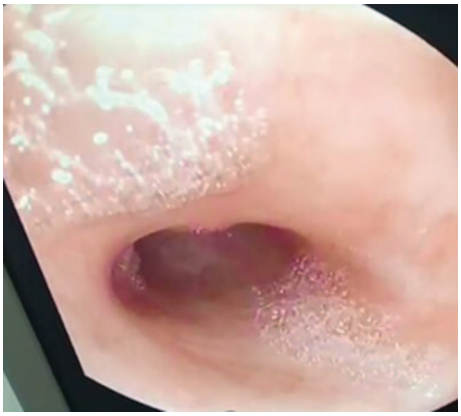
The child's condition remained stable, with persistent host-dominant chimerism (93.7% host cells). During interhospital periods, biologic therapy with adalimumab 40 mg every 2 weeks was maintained. Immunosuppressive therapy (azathioprine) was discontinued in August 2020.

Since August 2021, the patient has been followed at the gastroenterology department of the Federal Research and Clinical Center for Children and Adolescents, undergoing regular medical



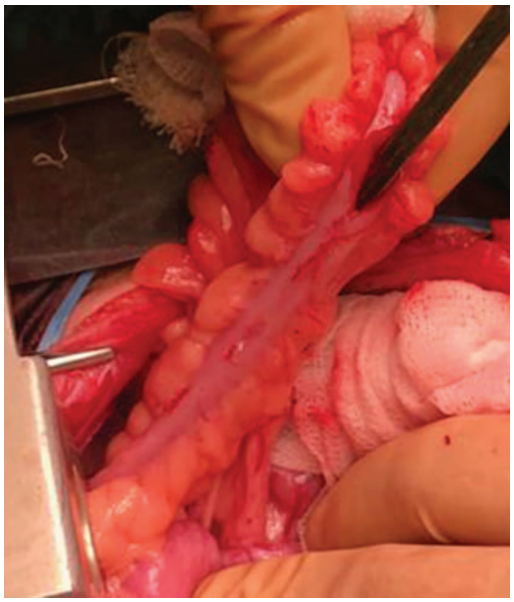
Clinical photo taken by the authors

Fig. 3. Peristomal complications: infected wound edges and dermatitis around the stoma



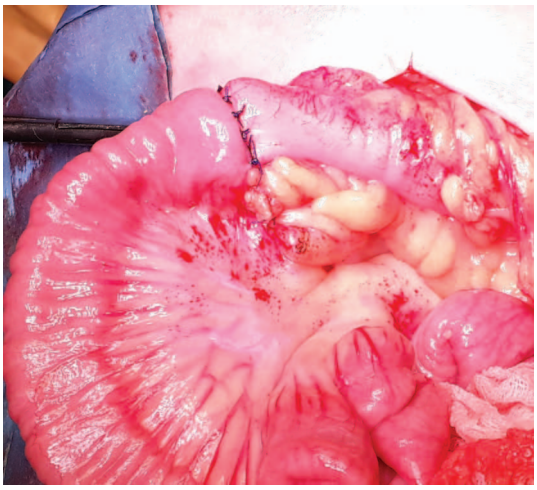
Clinical photo taken by the authors

Fig. 4. Endoscopic image (sigmoidoscopy data). Obliteration of the sigmoid colon lumen visualized endoscopically



Clinical photo taken by the authors

Fig. 5. Intraoperative photograph. View of the sigmoid colon almost obscured by "creeping fat": grooved probe inserted into the lumen up to the stricture



Clinical photo taken by the authors

Fig. 6. Intraoperative photograph. View of the direct ileo-ascendoanastomosis

examinations. Sustained clinical-endoscopic remission has been maintained. Control endoscopic examinations show:

- the ileo-ascending anastomosis remains inflammation-free and patent;
- terminal ileum appears normal;
- colonic mucosa is smooth with occasional stellate-shaped whitish scars.

In January 2022, the examination at Zurich Children's Hospital (Switzerland) recommended:

- continued immunoglobulin therapy;
- gradual immunosuppression tapering under gastroenterological supervision.

The current management includes:

- regular immunology consultations at the Dmitry Rogachev National Research Center;
- ongoing rituximab therapy for immune complication prophylaxis;
- maintenance adalimumab 40 mg biweekly SC for immune colitis;
- oral mesalazine 60 mg/kg/day;
- replacement immunoglobulin therapy (0.5 g/kg/month IV/SC) for antibody deficiency;
- prophylactic azithromycin courses for infection prevention.

DISCUSSION OF THE CLINICAL CASE

Very early-onset IBD (VEO-IBD) is a rare condition characterized by the disease onset before 6 years of age, occurring in 3–15% of pediatric IBD cases. It presents with severe clinical activity, refractoriness to therapy, and higher mortality rates [5, 9]. Conventional treatments for early-onset IBD, such as anti-TNF therapy, demonstrate limited efficacy [10]. In cases of VEO-IBD (particularly before 2 years of age) or when primary immunodeficiency (PID) warning signs are present [4, 7], diagnostic evaluation for monogenic mutations with PID verification should be performed [11].

We present a clinical case of monogenic Crohn's disease manifesting with intestinal symptoms since the neonatal period, perianal lesions, recurrent infections of various localizations in early childhood, atypical disease course, and refractoriness to standard medical therapy. The gastroenterologist awareness and vigilance enabled timely suspicion of PID and subsequent whole-exome sequencing, which revealed X-linked lymphoproliferative syndrome type 2 (XLP).

Among all PIDs, XLP2 most frequently exhibits IBD-like clinical presentation, with colonic involvement mimicking Crohn's disease occurring in 19% of children with XLP2 [12]. XLP results from mutations in the *XIAP* gene (X-linked inhibitor of apoptosis protein), also known as *BIRC4* (baculoviral IAP repeat-containing protein 4), leading to enhanced apoptosis of regulatory T-lymphocytes (NKT cells and CD^{4+} Tregs) and consequent marked pathogen-induced immune responses [13].

Beyond timely PID diagnosis, a key factor in successful management of monogenic Crohn's disease is surgical intervention with diverting stoma formation, aimed at controlling colonic inflammation and improving nutritional status. Currently, there are no international or national guidelines on indications for surgical intervention in refractory colitis, with only isolated publications supporting ileostomy creation to alleviate severe clinical symptoms in children with VEO-IBD and to enable stem cell transplantation [8, 14]. We concur with

international researchers regarding the importance of preventive ileostomy prior to HSCT to reduce potential complications and advocate for fecal diversion as part of staged management for severe refractory colitis with perianal involvement in Crohn's disease and Crohn's-like PID.

In our case, we demonstrated that elective preventive ileostomy ensured control of septic (infectious) complications and reduced intestinal risks during HSCT, despite episodes of graft rejection.

While stoma may temporarily alleviate symptoms, it cannot ensure long-term clinical remission in children with IBD. Moreover, the incidence of various peristomal complications increases over time [15]. In our case, we observed such complications one year after stoma formation (stoma evagination, bowel necrosis, and wound dehiscence following emergency surgery). Additionally, prolonged ileostomy with excluded colon may lead to "diversion colitis," requiring differentiation from immune-mediated colitis flare when treatment refractoriness develops. The etiology of the sigmoid colon stricture remains unclear — it could have resulted from chronic graft-versus-host reaction or Crohn's-like transmural colonic involvement.

Despite initial inefficacy of adalimumab and unsuccessful repeat HSCT, an individualized approach combining optimized medical therapy with symptomatic treatment and well-timed staged surgical interventions allowed us to achieve favorable outcomes in this child with severe PID manifesting Crohn's-like intestinal and perianal involvement.

CONCLUSION

Timely diagnosis of primary immunodeficiency (PID) enables prompt initiation of targeted therapy or hematopoietic stem cell transplantation (HSCT), which in most cases leads to disease remission and improves patients' quality and duration of life.

Optimal patient-centered management strategies for children with severe conditions like monogenic forms of inflammatory bowel disease (IBD) require:

- careful planning of surgical and therapeutic interventions;
- coordinated multidisciplinary team follow-up across specialized departments;
- continuous monitoring throughout all treatment phases.

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FUNCTIONAL ELECTRICAL STIMULATION FOR GAIT CORRECTION IN THE EARLY RECOVERY PHASE AFTER ISCHEMIC STROKE

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Introduction. Gait dysfunction is a complication of acute cerebrovascular accidents, which is biomechanically manifested as reduced speed and asymmetry in spatiotemporal and kinematic parameters. These impairments can be corrected using functional electrical stimulation (FES) of muscle contraction; however, the available literature primarily describes its application during the late recovery phase of stroke.
Objective. Evaluation of the potential of multichannel FES for gait recovery in early post-stroke rehabilitation.
Materials and methods. The study included 11 patients (2 females and 9 males) aged 46–66 years in the early recovery period after an ischemic stroke (time since stroke onset was 69.1 ± 52.0 days) and 34 healthy subjects (18 females and 16 males) as a control group. The lower limb muscle strength and tone were assessed using the Medical Research Council Scale for Muscle Strength and the modified Ashworth scale, respectively. Gait function was evaluated using the Dynamic Gait Index, Hauser Ambulation Index, Timed-Up-and-Go test, and 10-Meter Walk test. Gait pattern function (b770), obstacle negotiation (d4551), and short-distance walking (d4500) were also examined. All patients underwent a FES therapy course (mean number of sessions — 10.8). Clinical and biomechanical examinations were performed before and after the FES therapy course. Biomechanical gait analysis was conducted using a Stadis system (Neurosoft, Russia). Statistical analysis was performed using the Statistica 12.0 software.
Results. The conducted clinical evaluation demonstrated a minor yet statistically significant functional improvement in post-treatment testing. An increase in the scores of Dynamic Gait Index and 10-Meter Walk test was observed. A decrease in the values of Hauser Index values and the completion time of Timed- Up-and-Go test, as well as in domains (d770) and (d4500), was noted. Gait function showed improvement. The values of walking speed ($p < 0.05$), double support time on the paretic side ($p < 0.05$), and *m. gastrocnemius* activity on both the paretic and unaffected sides ($p < 0.05$) increased.
Conclusions. The observed changes in gait function were typical of hemiparesis. During the FES therapy course, the patients showed no negative reactions. The clinical and biomechanical gait functions of patients showed minor but positive changes during the FES therapy course. Among biomechanical parameters, the amplitude of the gastrocnemius muscle course on the paretic side significantly increased, which is one of the FES target parameters. Short courses of multichannel FES can be applied in this patient category; however, their effectiveness is insufficient. Approaches to improving the FES effectiveness require further investigation.

Keywords: ischemic stroke; paresis; gait; rehabilitation; electrical stimulation; gait biomechanics; hemiplegic gait

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ФУНКЦИОНАЛЬНАЯ ЭЛЕКТРИЧЕСКАЯ СТИМУЛЯЦИЯ ПРИ ХОДЬБЕ В РАННЕМ ВОССТАНОВИТЕЛЬНОМ ПЕРИОДЕ ИШЕМИЧЕСКОГО ИНСУЛЬТА

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Введение. Одним из осложнений острых нарушений мозгового кровообращения является нарушение функции ходьбы, которое биомеханически характеризуется снижением скорости и асимметрией пространственно-временных и кинематических параметров. Для коррекции данных изменений возможно применение функциональной электростимуляции мышц (ФЭС), однако в имеющейся литературе данный метод применяется преимущественно в позднем восстановительном периоде инсульта.
Цель. Оценка возможности применения многоканальной ФЭС у пациентов в раннем восстановительном периоде инсульта для восстановления функции ходьбы.
Материалы и методы. В исследовании приняли участие 11 пациентов (2 женщины и 9 мужчин) в возрасте от 46 до 66 лет в раннем восстановительном периоде ишемического инсульта (количество дней после инсульта составило 69,1 ± 52,0 дня) и 34 здоровых

испытуемых (18 женщин и 16 мужчин) — контрольная группа. Изучали: мышечную силу нижних конечностей по Medical Research Council Weakness Scale, мышечный тонус нижних конечностей по модифицированной шкале Ашфорт; функцию ходьбы оценивали по индексу динамической походки, индексу Хаузера, тестам «Встань и иди» и десятиметровой ходьбы; а также исследовали функцию стереотипа походки (d770), преодоление препятствий (d4551) и ходьбу на короткие расстояния (d4500). Всем пациентам проведен курс ФЭС (среднее количество — 10,8 процедуры). Клиническое и биомеханическое исследования выполнены до и по окончании курса ФЭС. Биомеханическое исследование ходьбы проведено с помощью комплекса программного обеспечения «Стэдис» («Нейрософт»). Статистическая обработка данных выполнена в программе Statistica 12.0.

Результаты. Клиническая оценка показала незначительное, но достоверное функциональное улучшение по результатам тестирования после проведенного лечения. Отмечено увеличение значений индекса динамической походки и теста 10-метровой ходьбы, уменьшение индекса Хаузера и времени выполнения теста «Встань и иди», а также по доменам (d770) и (d4500). Функция ходьбы улучшилась. Возросли значения скорости ходьбы ($p < 0,05$), увеличился период двойной опоры на паретичной стороне ($p < 0,05$), возросла активность *m. gastrocnemius* на паретичной и здоровой сторонах ($p < 0,05$).

Выводы. Обнаруженные изменения функции ходьбы были типичны для гемипареза. В ходе проведения курса ФЭС у пациентов не было выявлено негативных реакций на проводимую стимуляцию. Клинические и биомеханические функции ходьбы пациентов за время курса ФЭС изменилась незначительно, но динамика их положительная. Из биомеханических параметров достоверно возросла амплитуда икроножной мышцы на стороне пареза, что является одним из целевых параметров ФЭС. Проведение коротких курсов многоканальной ФЭС данной категории больных возможно, но недостаточно эффективно. Повышение эффективности ФЭС требует дальнейшего изучения.

Ключевые слова: ишемический инсульт; парез; ходьба; реабилитация; электростимуляция; биомеханика ходьбы; гемиплегическая ходьба

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INTRODUCTION

Acute cerebrovascular accident (ACVA) is the second leading cause of death and one of the main causes of disability worldwide [1, 2]. The incidence of strokes and the costs associated with necessary rehabilitation measures have been growing globally, including due to persistent health impairment experienced by a significant proportion of ACVA survivors [3].

The complications of ACVA can be distinguished into motor [4], cognitive [5], and sensory impairments [6]. One serious complication of motor disorders consists in an increased risk of falls [7] due to dorsoflexor weakness and the appearance of foot drop in the paretic lower limb [8]. A slow walking speed and asymmetry of lower limb movements are often observed, associated with reduced range of motion in the joints and the need to swing the leg sideways [9, 10, 11, 12]. In particular, spatial asymmetry is related to step length changes [13, 14].

Given the urgency of prompt restoration of motor functions in ACVA patients, improved rehabilitation methods are increasingly attracting the research attention. One such approach is functional electrical stimulation (FES) of muscle contraction.

Moe et al. described the FES method primarily in the context of performing a specific functional task [15], particularly walking [16]. A number of studies reported the effectiveness of FES in correcting typical gait changes in hemiparesis. However, the mechanism of this effect and the system for evaluating the results remain unclear. Most FES studies use changes in walking speed and muscle strength as criteria [17, 18]. Although being clinically significant, these criteria do not provide a detailed biomechanical understanding.

The authors [19] investigated the direct effects of FES on the gluteus medius and tibialis anterior muscles in post-stroke patients, including those using walking aids, and noted the importance of analyzing movements not only of the affected limb but also of the unaffected limb. Despite the findings, the researchers could not clarify the etiology of the increased step length in the unaffected limb. Another study [20] demonstrated the possibility of correcting knee hyperextension and foot drop with FES; however, the authors emphasized the need for further methodological development of this approach. Unfortunately, the current literature does not address the use of FES in patients during the early recovery period after a stroke.

In this study, we set out to assess the feasibility and to evaluate the outcomes of multichannel FES applied in patients during the early recovery period after a stroke for gait function correction.

MATERIALS AND METHODS

The study was conducted at the Scientific Research Center for Medical Rehabilitation of the Federal Center for Brain and Neurotechnologies from April to December 2024.

The study included patients with hemiparesis in the early recovery period after a first-ever ischemic stroke (< 180 days) in the middle cerebral artery territory, aged under 75 years, capable of independent ambulation (walking) without assistance, including with the use of walking aids (cane).

The exclusion criteria were cognitive impairments preventing patients from understanding instructions; sensorimotor aphasia; decompensated somatic pathology; diseases of the central and peripheral nervous system (except stroke) accompanied by neurological deficits (sequelae of trauma, tumors, polyneuropathies, etc.); orthopedic pathology (joint deformities, contractures, amputations, etc.); history of epileptic activity; skin diseases with rashes in electrode placement areas; patient refusal to participate.

Following screening, 11 patients (2 females and 9 males) aged 46 to 66 years (mean age 57.6 ± 8.0 years) were enrolled. Right-sided hemiparesis was observed in 4 participants. The mean time since stroke was 69.1 ± 52.0 days. The mean body mass index in the group was 24.9 kg/m^2.

Additionally, 34 healthy participants (18 females and 16 males) were included as a control group. The mean age of participants was 29.8 years, with a mean body mass index of 20.6 kg/m^2.

Clinical Status Assessment Methodology

For assessing the clinical status of patients, the following scales and scoring systems were used:

- Lower limb muscle strength was evaluated using the Medical Research Council Scale for Muscle Strength [21];
- Lower limb muscle tone was assessed with the Modified Ashworth Scale (mAS) [22].

The following instruments were applied for gait function evaluation: Dynamic Gait Index (DGI) [23], Hauser Ambulation Index [24], Timed-Up-and-Go Test (TUG) [25], 10 Meter Walk Test (10MWT) [26].

Health impairments and patient capabilities were assessed within the “Activity and Participation” domains of the International Classification of Functioning, Disability and Health [27, 28]: gait pattern function (b770), negotiating obstacles (d4551), short-distance walking (d4500).

Gait Function Assessment Methodology

Study Procedure

All patients underwent preliminary biomechanical gait analysis using a Stadis system (Neurosoft, Russia). Spatiotemporal and kinematic gait parameters were recorded using inertial sensors secured with elastic cuffs at the sacrum level and on both lower limbs: on the lateral surface of the mid-thigh, at the lateral malleolus of the ankle joint, and on the dorsal foot surface. Simultaneously, electromyographic (EMG) activity of lower limb muscles was recorded (each sensor included two EMG channels) via electrodes placed at the mid-length of:

- *m. quadriceps femoris,*
- hamstrings (*m. biceps femoris, m. semitendinosus, m. semimembranosus*),
- *m. tibialis anterior,*
- *m. gastrocnemius (both heads).*

During testing, patients walked at a self-selected pace along an 8.5-m straight path with turns at the end. Biomechanical data were recorded until 30 gait cycles had been achieved. The software automatically excluded unstable steps (turns, stumbling, acceleration/deceleration). The output included:

- spatiotemporal gait cycle parameters,
- kinematic data as joint angle curves (flexion/extension during the gait cycle) for hip, knee, and ankle joints,
- muscle EMG activity profiles (envelope EMG).

The first biomechanical assessment was performed for both patient and healthy control groups (baseline); the second assessment was conducted only for patients after FES therapy.

Recorded Parameters

Temporal (gait cycle [GC] in sec; others as % of GC):

- stance phase (ST, %),
- single support phase (SS, %),
- double support phase (DS, %),
- the beginning of the terminal double limb stance phase (BTDLS, %).

Spatial:

- foot clearance (cm),
- circumduction (cm),
- walking speed (km/h).

Kinematic: angular range of motion (maximum flexion/extension, °) with temporal phase (% of GC).

Hip joint (H):

- amplitude and phase of initial flexion (Ha1 and Hx1, respectively),
- extension during late-stance (Ha2, Hx2),
- flexion during swing (Ha3, Hx3).

Knee joint (K):

- initial amplitude (K0),
- amplitude and phase of first flexion (Ka1, Kx1),
- amplitude and phase of first extension (Ka2, Kx2),
- amplitude and phase of second flexion (Ka3, Kx3).

Ankle joint (AJ):

- initial amplitude (A0),
- amplitude and phase of first dorsiflexion (Aa1, Ax1),
- amplitude and phase of first plantarflexion (Aa2, Ax2),
- amplitude and phase of second dorsiflexion (Aa3, Ax3),
- amplitude and phase of second plantarflexion (Aa4, Ax4).

EMG activity (peak amplitude [μV] and phase [%GC]):

- *m. tibialis anterior* (TA): two peaks (TAa1, TAa2) with phases (TAx1, TAx2),
- *m. gastrocnemius* (GSC): one peak (GSCa) and phase (GSCx),
- *m. quadriceps femoris* (QF): two peaks (QFa1, QFa2) with phases (QFx1, QFx2), respectively
- hamstrings (HM): one peak (HMa) and phase (HMx).

Recorded goniograms and envelope EMG (muscle activation profile) parameters are illustrated in Fig. 1.

Functional Electrical Stimulation (FES) Methodology

For the FES procedure, we used stimulation devices from a Stedis system (Neurosoft, Russia), and FIAB stimulation electrodes (Italy). The devices were secured with the same elastic cuffs as those used for biomechanical gait analysis, positioned on: the sacrum, thighs, and external malleoli. Stimulation electrodes were applied to the muscles of the paretic limb at the upper and lower thirds of *mm. quadriceps femoris*, *tibialis anterior*, *gastrocnemius* and hamstring (Fig. 2).

At the next stage, the current intensity was adjusted based on two criteria: test stimulation had to produce visible muscle contraction, while the patient's sensations had to remain below their pain threshold. The current intensity (stimulation strength) was set at the beginning of each session for each stimulated muscle. The current frequency and pulse duration parameters remained unchanged, i.e., 50 Hz and 200 ms, respectively.

After determining the current intensity, the system was calibrated and the training was initiated. Patients walked in a straight line at a self-selected pace, making turns at the end of the path and continuing to walk. Electrical pulses were delivered to the muscles at specific points in the gait cycle corresponding to the physiological peak of muscle bioelectric activity during walking in healthy individuals. Specifically:

- for *m. quadriceps femoris* — pulses were delivered at the beginning of the stance phase and at the end of the swing phase;
- for hamstring — at the beginning of the stance phase and the end of the swing phase;
- for *m. tibialis anterior* — at the beginning of the stance phase and in the middle of the swing phase;
- for *m. gastrocnemius* — in the middle of the gait cycle.

The patient continued walking for 30 min, after which the training session ended. The procedure was stopped

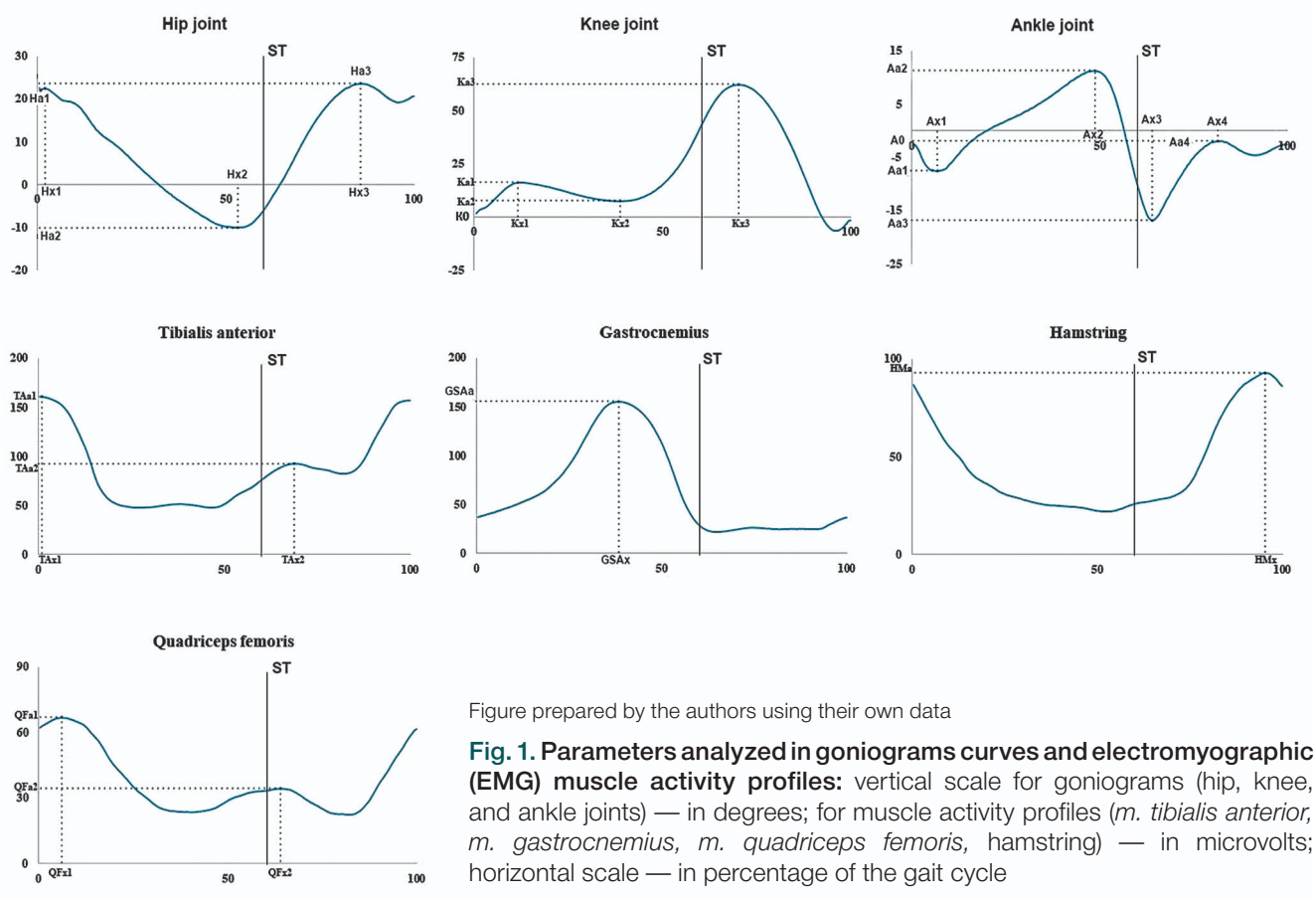

Figure prepared by the authors using their own data

Fig. 1. Parameters analyzed in goniograms curves and electromyographic (EMG) muscle activity profiles: vertical scale for goniograms (hip, knee, and ankle joints) — in degrees; for muscle activity profiles (*m. tibialis anterior, m. gastrocnemius, m. quadriceps femoris,* hamstring) — in microvolts; horizontal scale — in percentage of the gait cycle

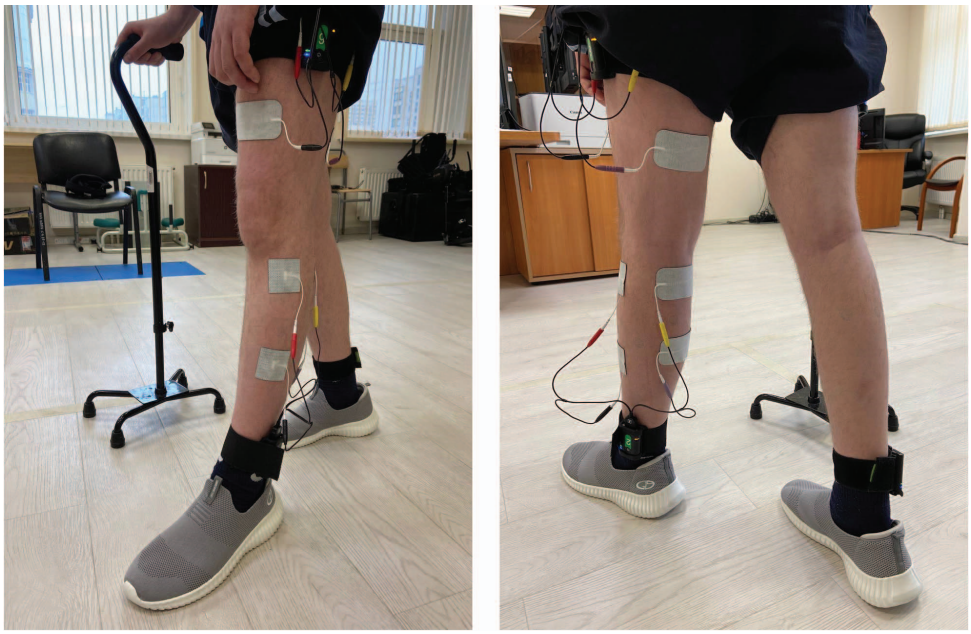

Photo taken by the authors

Fig. 2. Placement of stimulation electrodes and devices on the patient's lower limb: the electrodes were placed on the hemiparetic side, while the devices were attached to both legs to record biomechanical parameters during stimulation

earlier if subjective complaints appeared (dizziness, fatigue) or at the patient's request. Procedures were performed daily, five times per week. The course duration was determined by the patients' hospital stay and averaged 10.8 procedures. The average procedure duration was 25.5 min.

Data Statistical Processing

For statistical data processing, we used the Statistica 12.0 software (StatSoft, Tulsa, USA). The normality of quantitative parameter distributions was assessed using the Shapiro–Wilk test, which showed non-normal distributions ($p < 0.05$); therefore, all data were presented as medians with first and third quartiles Me [Q1; Q3]. To compare walking parameters in patients before and after the FES course, we used the Wilcoxon test. To compare walking parameters between the patient and control groups, we applied the Mann–Whitney U-test. A p-value < 0.05 was considered statistically significant.

RESULTS

Clinical parameters

The comparison of clinical characteristics in the patient group before and after the FES course revealed statistically significant changes indicating improved walking function (Table 1):

- Dynamic Gait Index increased by 3 points;
- Hauser Ambulation Index decreased by 1 point;
- Timed-Up-and-Go test improved by 7 s;
- 10-Meter Walk test speed increased by 0.15 m/s.

ICF (International Classification of Functioning) domains:

- gait pattern function (b770) decreased by 1 point,
- short-distance walking (d4500) decreased by 1 point.

Spatial and temporal parameters

The comparison of parameters before and after the FES course revealed the following statistically significant changes (Table 2):

- Increased double support time on the paretic side,
- Decreased double support time on the unaffected side,
- Improved walking speed.

The comparison of pre- and post-FES parameters with control group values revealed the following statistically significant differences:

- extended gait cycle duration (GC),
- increase in the stance phase (ST) duration on the unaffected side,
- increased paretic-side single support (SS) phase,
- increase in double support (DS) phase bilaterally,
- earlier beginning of the terminal double limb stance phase (BTDLS) on the paretic side,
- delayed beginning of the terminal double limb stance phase (BTDLS) on the unaffected side,
- reduced foot clearance on the paretic side,
- increased circumduction on the paretic side,
- significantly slower walking speed in the patient group compared to healthy controls.

Table 1. Clinical parameters before and after the functional electrical stimulation (FES) course

Clinical Parameter	Before FES	After FES
Lower-extremities muscle strength, score	3	3
Clinical scales and tests		
Lower-extremities muscle tone on Modified Ashworth Scale, score	1–2	1–2
Dynamic Gait Index	16 [14; 17]	19* [18; 20]
Hauser Ambulation Index	4 [3; 4]	3* [3; 4]
Timed-Up-and-Go test, s	32 [25; 36]	25* [19; 30]
10-Meter Walk test, m/s	0.75 [0.7; 0.8]	0.9* [0.85; 1]
ICF categories		
b770 — gait pattern functions	2 [2; 3]	1* [1; 2]
d4551 — obstacle negotiation	2 [1; 2]	1 [1; 2]
d4500 — short-distance walking	2 [1; 2]	1* [0; 1]

Table compiled by the authors based on their own data

Note: * — statistically significant changes, $p < 0.05$.

Table 2. Spatiotemporal parameters before and after the functional electrical stimulation (FES) course

Parameter	Before FES course		After FES course		Control group
	Paretic side	Unaffected side	Paretic side	Unaffected side	
GC, s	1.6 [1.5; 2]*	1.6 [1.4; 1.9]*	1.5 [1.4; 2]*	1,5 [1,4; 2]*	1,1 [1,1; 1,2]
ST (%)	63.3 [60.8; 64.5]	74.2 [69.1; 78]*	62.1 [59.9; 65]	71,8 [67,9; 78,2]*	63,1 [62,4; 64,4]
SS (%)	26.3 [22.2; 31.2]*	36.9 [35.9; 39.5]	27.6 [21.5; 31.7]*	37,8 [35,2; 39,7]	36,9 [35,7; 37,9]
DS (%)	34.5 [30.6; 43]*	34.8 [30.7; 42.8]*	35 [27.6; 40.8]* #	34,4 [28,2; 41,4]* #	26,1 [24,6; 28,1]
BTDLS (%)	41.6 [40.8; 45.8]*	57.1 [53.5; 60]*	42.8 [40; 45.6]*	56,4 [54,1; 60,1]*	49,9 [49,6; 50,3]
Foot clearance (cm)	8 [7; 12]*	13 [11; 15]	9 [7; 12]*	14 [11; 14]	13,5 [12; 15]
Circumduction (cm)	4 [3; 6]*	2 [2; 4]	4 [3; 6]*	2 [2; 3]	3 [2; 4]
Walking Speed (km/h)	1.7 [1.2; 2.5]*		2.2 [1.3; 2.4]*#		4.3 [4; 5]

Table compiled by the authors based on their own data

Note: * — significant differences versus controls, $p < 0.05$; # — pre-post differences in ipsilateral parameters reached statistical significance, $p < 0.05$; GC — gait cycle; ST — stance phase; SS — single support phase; DS — double support phase; BTDLS — the beginning of the terminal double limb stance phase.

Table 3. Kinematic parameters before and after the functional electrical stimulation (FES) course

Location	Parameter	Before FES course		After FES course		Control group
		Paretic side	Unaffected side	Paretic side	Unaffected side	
Hip Joint	Ha1	15* [9; 16]	23 [19; 30]	15* [10; 17]	24 [20; 28]	23 [20; 25]
	Hx1	2 [1; 5]	1* [1; 2]	3 [1; 7]	2 [1; 5]	2 [2; 3]
	Ha2	-6* [-9; 1]	-6* [-10; -3]	-8* [-11; -2]	-7*# [-11; -3]	-11 [-12; -9]
	Hx2	50* [47; 55]	59* [56; 64]	50* [47; 52]	61* [57; 66]	53 [51; 55]
	Ha3	16* [11; 28]	31* [26; 34]	17* [16; 27]	31* [25; 32]	24 [22; 27]
	Hx3	90 [84; 92]	90* [86; 93]	88# [83; 91]	89 [87; 93]	87 [84; 89]
Knee Joint	K0	2 [0; 4]	12* [8; 15]	1 [-3; 5]	10* [7; 13]	3 [-1; 5]
	Ka1	10* [4; 12]	14 [14; 20]	10* [3; 11]	16 [13; 19]	17 [14; 19]
	Kx1	8* [7; 10]	9* [7; 12]	11* [8; 13]	10# [7; 15]	12 [12; 14]
	Ka2	2* [-4; 9]	6 [4; 9]	-1* [-4; 2]	5 [2; 11]	6 [4; 9]
	Kx2	33* [31; 37]	38 [34; 43]	37 [32; 42]	38 [35; 40]	37 [34; 41]
	Ka3	35* [27; 52]	61 [56; 62]	37* [30; 47]	61 [59; 64]	63 [60; 67]
	Kx3	70 [66; 73]	79 [74; 83]*	71 [64; 73]	77* [74; 81]	70 [69; 71]
Ankle Joint	A0	-9* [-12; -2]	-4 [-5; -3]	-10* [-15; -6]	-3 [-4; -1]	-3 [-5; 0]
	Aa1	-11 [-14; -5]	-7 [-9; -4]	-14* [-15; -13]	-7 [-10; -5]	-8 [-10; -6]
	Ax1	4* [1; 5]	4* [3; 7]	3* [1; 6]	6# [3; 8]	7 [6; 8]
	Aa2	9 [5; 14]	10 [9; 12]	8 [5; 12]*	13 [10; 14]	12 [10; 15]
	Ax2	49 [47; 51]	58* [56; 60]	48.75 [48; 50]	57* [56; 59]	48 [46; 50]
	Aa3	-5* [-11; -3]	-9* [-18; -7]	-10* [-13; -7]	-15* # [-17; -12]	-19 [-22; -15]
	Ax3	74* [66; 79]	74* [71; 80]	67* [65; 76]	73* [70; 77]	64 [63; 65]
	Aa4	-9* [-11; -3]	-4* [-10; -4]	-9* [-14; -5]	-6* [-9; -3]	-1 [-3; 1]
	Ax4	94* [93; 98]	82 [81; 98]	99* [95; 100]	81 [81; 97]	86 [81; 97]

Table compiled by the authors based on their own data

Note: * — significant differences versus controls, $p < 0.05$; # — pre-post differences in ipsilateral parameters reached statistical significance, $p < 0.05$; Ha1 and Ha2 — amplitude and phase of initial flexion; Ha2 and Hx2 — extension during mid-stance; Ha3 and Hx3 — flexion during swing; K0 — initial amplitude of knee; Ka1 and Kx1 — amplitude and phase of initial flexion; Ka2 and Kx2 — amplitude and phase of first extension; Ka3 and Kx3 — amplitude and phase of second flexion; A0 — initial amplitude of ankle; Aa1 and Ax1 — amplitude and phase of first dorsiflexion; Aa2 and Ax2 — amplitude and phase of first plantarflexion; Aa3 and Ax3 — amplitude and phase of second dorsiflexion; Aa4 and Ax4 — amplitude and phase of second plantarflexion.

Kinematic parameters

The comparison of pre- and post-FES parameters revealed the following statistically significant ($p < 0.05$) changes (Table 3):

- earlier Hx3 on the paretic side,
- increased Ha2 on the unaffected side,
- delayed Kx1 on the unaffected side,
- delayed Ax1 on the unaffected side,
- increased Aa3 on the unaffected side.

The comparison of pre-FES patient parameters with healthy controls revealed statistically significant differences ($p < 0.05$). Thus, the patients demonstrated reduced amplitude of initial hip flexion (Ha1) on the paretic side; earlier onset of this flexion (Hx1) on the unaffected side; decreased extension amplitude (Ha2) bilaterally with earlier phase onset (Hx2) on the paretic side and delayed onset on the unaffected side; reduced swing-phase flexion amplitude (Ha3) on the paretic side with increased amplitude on the unaffected side; and delayed onset of this flexion phase (Hx3) on the unaffected side.

In the knee joint, the analysis revealed significant kinematic alterations, i.e., reduced amplitude of first flexion (Ka1) on the paretic side accompanied by earlier onset of this flexion phase (Kx1) bilaterally; decreased extension amplitude (Ka2) with premature phase initiation (Kx2) on the paretic side; reduction in flexion amplitude (Ka3) on the paretic limb coupled with delayed flexion onset (Kx3) on the unaffected side.

In the ankle joint, earlier onset of the first extremum phase (Ax1) bilaterally and delayed initiation of full flexion (Ax2) on the unaffected side; reduced amplitude (Aa3) bilaterally with delayed phase onset (Ax3) on both sides; increased amplitude (Aa4) bilaterally and delayed initiation of its phase (Ax4) on the paretic side were noted.

The comparative analysis of kinematic parameters in post-FES patients versus healthy controls revealed the following statistically significant changes ($p < 0.05$):

Hip joint

- decreased amplitude of initial flexion (Ha1) on paretic side,

Table 4. Electromyographic parameters before and after functional electrical stimulation (FES)

Muscle	Parameter	Before FES course		After FES course		Control group
		Paretic side	Unaffected side	Paretic side	Unaffected side	
TA	TAa1	72* [33; 95]	163 [134; 230]	69* [58; 135]	208* [178; 278]	159 [118; 186]
	TAx1	58* [4; 60]	10* [9; 28]	60* [12; 60]	20* [9; 26]	1 [1; 2]
	TAa2	70* [58; 104]	143 [118; 215]	71* [60; 139]	180 [136; 222]	154 [116; 185]
	TAx2	68* [62; 97]	100 [84; 100]	66* [64; 100]	100 [84; 100]	99 [98; 100]
GSC	GSCa	50* [27; 81]	145 [133; 163]	70* # [54; 96]	171# [164; 208]	154 [113; 202]
	GSCx	31* [28; 39]	44* [35; 47]	37 [32; 40]	39 [35; 47]	39 [37; 40]
QF	QFa1	62 [41; 67]	92* [72; 109]	62 [52; 84]	89 [67; 174]	63 [41; 86]
	QFx1	13* [10; 17]	21* [6; 24]	14 [6; 16]	12* [9; 23]	7 [5; 9]
	QFa2	40 [31; 58]	75 [60; 126]	48* [40; 81]	82 [60; 116]	57 [39; 81]
	QFx2	100 [51; 100]	97 [51; 100]	100 [99; 100]	95 [52; 100]	100 [99; 100]
HM	HMa1	53* [43; 71]	108 [83; 146]	59* [40; 79]	129* [115; 146]	83 [62; 123]
	HMx1	13* [10; 19]	26 [12; 56]	14 [11; 25]	45 [31; 65]	92 [43; 95]

Table compiled by the authors based on their own data

Note: * — significant differences versus controls, $p < 0.05$; # — pre-post differences in ipsilateral parameters reached statistical significance, $p < 0.05$; TA — *m. tibialis anterior*; GSC — *m. gastrocnemius*; QF — *m. quadriceps femoris*; HM — hamstrings.

- decreased extension amplitude (Ha2) bilaterally,
- earlier extension phase onset (Hx2) on paretic side,
- delayed extension phase onset (Hx2) on unaffected side,
- decreased flexion amplitude (Ha3) on paretic side,
- increased flexion amplitude (Ha3) on unaffected side.

Knee joint
- decreased initial flexion amplitude (Ka1) on paretic side,
- earlier initial flexion onset (Kx1) on paretic side,
- decreased amplitudes (Ka2 and Ka3) on paretic side,
- delayed termination of second flexion phase (Kx3) on unaffected side.

Ankle joint
- increased first amplitude (Aa1) on paretic side,
- earlier first phase onset (Ax1) on paretic side,
- decreased second amplitude (Aa2) on paretic side,
- delayed second phase onset (Ax2) on unaffected side,
- decreased third amplitude (Aa3) bilaterally,
- delayed third phase onset (Ax3) bilaterally,
- increased fourth amplitude (Aa4) bilaterally,
- delayed fourth phase onset (Ax4) on paretic side.

Electromyographic parameters

The comparison of muscle bioelectrical activity profiles before and after the FES course revealed two statistically significant changes ($p < 0.05$): an increase in the peak activity of the gastrocnemius muscle was observed for both the paretic and unaffected sides (Table 4).

The comparative analysis of pre-FES electromyographic parameters between patients and healthy controls revealed the following statistically significant differences ($p < 0.05$):

m. tibialis anterior
- decreased TAa1 amplitude on paretic side,
- delayed TAx1 onset bilaterally,
- reduced TAa2 amplitude on paretic side,
- earlier TAx2 onset on paretic side,

m. gastrocnemius
- reduced GSCa amplitude on paretic side,
- earlier GSCx onset on paretic side,
- delayed GSCx onset on unaffected side,

m. quadriceps femoris
- increased QFa1 amplitude on unaffected side,
- delayed QFx1 onset bilaterally,
- increased QFa2 amplitude on unaffected side,

Hamstring muscles
- reduced HMa amplitude on paretic side,
- earlier HMx onset on paretic side.

The comparative analysis of post-FES electromyographic profiles between patients and healthy controls revealed the following statistically significant differences ($p < 0.05$):

m. tibialis anterior:
- decreased TAa1 amplitude on paretic side,
- increased TAa1 amplitude on unaffected side,
- delayed TAx1 onset bilaterally,
- reduced TAa2 amplitude on paretic side,
- earlier TAx2 onset on paretic side,

m. gastrocnemius:
- reduced GCa amplitude on paretic side,
- *m. quadriceps femoris*:
- delayed QFx1 onset on unaffected side,

Hamstring muscles:
- decreased HMa amplitude on paretic side,
- increased HMa amplitude on unaffected side.

DISCUSSION

Our study revealed minor yet characteristic gait alterations in patients with stroke-associated hemiparesis.

Following the FES therapy course, we observed:
- increased Dynamic Gait Index scores,
- improved 10-Meter Walk test performance,
- decreased Hauser Ambulation Index values,
- reduced Timed Up-and- Go (TUG) test completion time.

These outcome measures (10-Meter Walk and TUG) represent the most frequently reported FES efficacy parameters in literature [29, 30, 31], with our results being consistent with existing data. However, other studies have incorporated additional clinical measures showing more variable outcomes.

The systematic review by Wang et al. covering 14 studies with 945 hemiparetic patients demonstrated FES-induced improvements in:
- Fugl-Meyer Assessment (FMA) scores,
- Berg Balance Scale (BBS),
- 10-Minute Walk test,
- Modified Barthel Index (MBI),
- Functional Walking Test (FWT) [30].

Conversely, an eight-week FES trial (40 min/day, 5 days/week, n=92) by Matsumoto et al. failed to show statistically significant changes in 10-Meter Walk test, Fugl-Meyer Assessment, and Timed-Up-and-Go test [32].

The changes in spatiotemporal gait parameters observed in patients prior to the FES course demonstrated alterations characteristic of this post-stroke period. These included:
- increased gait cycle duration (GC),
- normal stance phase (SP) duration on the paretic side with prolonged SP on the unaffected side,
- reduced single support time (SS) on the paretic side with increased SS on the unaffected side,
- increased total double support time (DS),
- the beginning of the terminal double limb stance phase (BTDLS) timing (earlier on paretic, delayed on unaffected side),
- decreased foot clearance on the paretic side.

These biomechanical changes have been previously described [9, 10, 11, 12] and represent typical hemiparetic gait patterns.

Following FES intervention, we observed:

1. A minor yet statistically significant increase in the double support time bilaterally ($p < 0.05$) — a compensatory mechanism to improve body balance, as stability increases when both limbs are weight-bearing.
2. Increased walking speed, correlating with findings from other studies [33, 34].

As a rule, patients with hemiparesis also exhibit kinematic changes: reduced range of motion in the hip, knee, and ankle joints on the paretic side. In this case, the ankle joint is in slight extension, which reduces clearance and leads (along with other changes) to increased circumduction. The paretic side is characterized by reduced range of motion in the joints. At the same time, the healthy side is forced to compensate for the reduced activity of the paretic side. Thus, at low walking speeds, even normative kinematic parameters of the unaffected side already represent hyperfunction. The later peaks of several amplitudes on the unaffected side are the result of increased SP. The overall duration of stance phase increases, and thus the amplitude peaks also shift and occur later in time.

Following the FES course, only minor kinematic changes were observed, primarily on the unaffected side. In the available literature, FES is most commonly used for post-stroke patients with foot drop; consequently, kinematic changes are typically limited to the ankle joint. For instance, Güzel et al. described the positive effects of a four-week FES course on the ankle joint range of motion in patients during the early recovery phase after an ischemic stroke [34].

The EMG analysis revealed characteristic hemiparetic changes, including reduced activity amplitudes in all analyzed muscles on the paretic side. However, less pronounced changes were noted in QF compared to other muscle groups, both in terms of amplitude and activation profile [12, 35]. This particular muscle provides knee joint stability, and significant alterations in its activity make weight-bearing on the paretic limb impossible.

The rehabilitation course resulted in only one significant change: an increased GSC amplitude was observed bilaterally. Nevertheless, GSC activity on the paretic side remained more than two times lower than on the unaffected side, both before and after FES.

Our results demonstrate that during the early recovery period after a cerebral stroke, a three-week rehabilitation course in general and with FES application in particular objectively led to minor functional improvements. The FES training was conducted daily, with patients walking the maximum duration until fatigue. Stimulation intensity was also maintained at the maximum tolerable level for each patient. According to foreign researchers, FES courses are typically conducted over longer periods [36]. However, under the conditions of our study, exceeding 10 procedures proved particularly challenging. This limitation was previously noted in our earlier research into gait restoration using biofeedback methods [11, 37].

The duration of rehabilitation measures for patients with CNS impairments depends on their Rehabilitation Routing Scale (RRS) score, which reflects the degree of functional limitations and dependence on assistance for daily activities. According to the Program of State Guarantees for Free Medical Care, patients with RRS score 4 receive 14-day rehabilitation courses, while those with RRS score 5 receive 20-day courses. Typically, gait training begins for patients with RRS level 4 functional limitations, implying the actual length of rehabilitation between 10–12 days. Objective gait assessment is performed upon admission and before discharge.

Our findings indirectly confirm the insufficient duration of CNS rehabilitation courses within the current medical rehabilitation system and highlight the need for further investigation.

CONCLUSIONS

All patients included in this study demonstrated typical gait impairments associated with hemiparesis during the early recovery phase after a stroke. The administered course of multichannel FES revealed no adverse reactions. While clinical and biomechanical improvements during the FES course were modest, a statistically significant increase in gastrocnemius muscle amplitude was observed on the paretic side.

Our findings indicate that multichannel FES can be safely implemented for gait correction during early stroke recovery. However, the short 10-session daily treatment protocol failed to produce substantial functional improvements in the stimulated muscles.

Future research should focus on developing FES parameter adjustment methodologies tailored to specific biomechanical impairments, potentially enhancing therapeutic outcomes for gait rehabilitation in patients during the early recovery phase after an ischemic stroke.

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INFORMATIVITY ASSESSMENT OF WEBSITES OF TERRITORIAL ATTESTATION COMMISSIONS ON THE ATTESTATION PROCEDURE FOR HEALTHCARE SPECIALISTS

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Introduction. Due to the current decline in medical professionals' interest in the institution of qualification categories, the problem of improving the quality of information content and its distribution among the target audience becomes particularly relevant.
Objective. Quality assessment of the website informativity of territorial attestation commissions and public health administrations of the Russian Federation (RF) subjects regarding the assignment of qualification categories.
Materials and methods. The information search about the procedure for attesting healthcare specialists was carried out through 47 websites of the healthcare executive authorities of 83 RF subjects and territorial attestation commissions. A remote survey of 47 medical professionals was conducted: 25 (53.2%) men and 22 (46.8%) women (the average age of respondents was 32.3 ± 4.94 years) with a work experience in the specialty of two years. Each respondent reviewed the information about the attestation procedure posted on the websites of three different RF subjects. The websites were distributed among respondents randomly using an online random number generator. The survey was conducted using a questionnaire developed by the Department of Economics and Marketing at the Academy of Postgraduate Education of the Federal Medical and Biological Agency of Russia. Statistical analysis was performed using the SPSS software (IBM Company).
Results. In total, 47 (56.6%) websites of territorial attestation commissions contained information on all points of the Order of Ministry of Public Health of the Russian Federation (No. 458n dated August 31, 2023), regarding the rules and procedure for submitting documents. In the survey, the information sufficiency on the rules and procedure for attestation of medical professionals was rated higher (3.13 ± 1.04 points) compared to the information clarity (2.98 ± 1.02 points) (p = 0.009). The respondents' scores of the sufficiency and clarity of information on different websites differed significantly: $102.155 \le \chi^2 \le 110.978$ ($p \le 0.001$); for the same websites, the scores were identical (p = 0.881 and p = 0.976).The scores of information sufficiency and clarity did not depend on the respondents' age (p = 0.416 and p = 0.706), gender (p = 0.163 and p = 0.148), or profession (p = 0.901 and p = 0.947), or their work in organizations that provide care in different settings (p = 0.956 and p = 0.983).
Conclusions. The information about the attestation procedure of medical professionals, which is available on the websites of the public health authorities of RF subjects and on the websites of the respective territorial attestation commissions, needs to be corrected and updated.

Keywords: certification; qualification category; certification commission; information content

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ОЦЕНКА ИНФОРМАТИВНОСТИ САЙТОВ ТЕРРИТОРИАЛЬНЫХ АТТЕСТАЦИОННЫХ КОМИССИЙ О ПРОЦЕДУРЕ АТТЕСТАЦИИ РАБОТНИКОВ ЗДРАВООХРАНЕНИЯ

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Введение. Совершенствование информационного контента необходимо для решения проблем целевой аудитории и привлечения потенциальных пользователей в связи со снижением интереса врачей к институту присвоения квалификационных категорий.
Цель. Оценка качества информационного контента сайтов территориальных аттестационных комиссий и органов управления здравоохранением субъектов Российской Федерации относительно присвоения квалификационных категорий.
Материалы и методы. Выполнен поиск информации о процедуре аттестации медицинских работников на 47 сайтах органов исполнительной власти 83 субъектов Российской Федерации в сфере здравоохранения и сайтах территориальных аттестационных комиссий. Проведен заочный опрос 47 врачей: (25 (53,2%) мужчин и 22 (46,8%) женщин; средний возраст респондентов 32,30 ± 4,94 года) со стажем работы по специальности от 2 лет. Каждый респондент рассматривал информацию о прохождении аттестации, размещенную на сайтах трех различных субъектов Российской Федерации. Распределение сайтов по респондентам произведено случайным образом с помощью онлайн-ресурса генератора случайных чисел. Опрос проведен с использованием анкеты, которая была разработана сотрудниками кафедры экономики и маркетинга Академии постдипломного образования ФГБУ ФНКЦ ФМБА России. Статистическая обработка выполнена в программе SPSS (IBM Company).

Результаты. Информацию по всем пунктам приказа Минздрава России от 31 августа 2023 г. № 458н, касающимся правил и порядка подачи документов, содержали 47 (56,6%) сайтов территориальных аттестационных комиссий. При опросе достаточность информации сайтов о правилах и порядке аттестации врачами оценена выше (3,13 ± 1,04 балла) по сравнению с понятностью контента (2,98 ± 1,02 балла) ($p = 0,009$). Оценки респондентов достаточности и понятности контента разных сайтов значимо отличаются: $102,155 \le \chi^2 \le 110,978$ ($p \le 0,001$), а для одних и тех же сайтов — одинаковы ($p = 0,881$ и $p = 0,976$). Оценки достаточности и понятности информации не зависели от возраста респондентов ($p = 0,416$ и $p = 0,706$), их пола ($p = 0,163$ и $p = 0,148$), специальности ($p = 0,901$ и $p = 0,947$), работы в организациях, оказывающих помощь в разных условиях ($p = 0,956$ и $p = 0,983$).
Выводы. Информация о процедуре аттестации медицинских работников, размещенная на сайтах органов исполнительной власти субъектов Российской Федерации в сфере здравоохранения и сайтах территориальных аттестационных комиссий, нуждается в коррекции и актуализации.

Ключевые слова: аттестация; квалификационная категория; аттестационная комиссия; информационный контент

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INTRODUCTION

The quality of information systems determines the efficiency of organizations. This requires permanent improvement of such systems [1], including the quality of information content [2].

The website content is information intended for the target audience to meet their requirements within a specific field of activity [3]. The study [4] of web queries showed that information search accounts for 80% of all web operations. At the same time, the main deficiency of information content is known to be the overabundance of irrelevant and insignificant information [5]. A serious difficulty faced by developers of information content consists in recognizing the usefulness of the material and selecting targeted information [6].

The quality of information content is acquiring particular importance in the context of the growing amount of online information, leading to information overload and social media fatigue [7, 8]. Information content that motivates Internet users to take certain actions or influences their behavioral intentions is the “golden fleece” of any organization [9, 10]. Convincing information content is capable of changing the users’ attitudes and encouraging them to interact with the offeror [11, 12].

Due to the current decline in medical professionals’ motivation and interest in the qualification institution, their unwillingness to engage in additional work-related activities, fear of reducing the amount of free time, lack of understanding of all certification aspects and processes, as well as the desire to avoid complex procedures, the information content of the websites of certification organization should be as useful and convincing as possible [13, 14].

The Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023) “On Approval of the Procedure and Terms for Medical and Pharmaceutical Professionals to Obtain a Qualification Grade”[1] requires governmental agencies and organizations that have established attestation commissions to post information about these commissions, visiting hours, and document submission order, as well as the composition of expert groups, on their official websites.

In this study, we set out to assess the quality of information content on the websites of the healthcare authorities of the constituent entities of the Russian Federation (RF subjects) and the respective territorial certification commissions regarding the assignment of qualification categories.

MATERIALS AND METHODS

An information search about the procedure for certifying medical professionals on the websites of the healthcare executive authorities of 83 RF subjects and on the websites and territorial attestation commissions was carried out.

We evaluated the information availability on the websites of territorial certification commissions, as specified in Table 1. Information from paragraphs 34.1–34.8, 37, and 39 describes the rules for submitting documents for certification, while paragraphs 35 and 36 describe the procedure for submitting them. The information availability from the Order on the website[2] was evaluated positively (“yes”) provided that it met all the requirements of the relevant clause in the regulatory document.

A survey was conducted in absentia among 47 medical professionals with at least two years of relevant

[1] Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023) “On Approval of the Procedure and Terms for Medical and Pharmaceutical Professional to Obtain a Qualification Grade”.
[2] Ibid.

professional experience, who were enrolled in the Postgraduate Medical Education Academy. The survey focused on 47 websites identified during the initial assessment as containing information about the rules and procedures for submitting documents for certification. Each respondent reviewed the certification information posted on the websites of three different RF subjects. Thus, the information on each website was evaluated independently by three respondents. The corresponding website links were sent to the respondents via email. The distribution of websites among the respondents was carried out in a random manner using the Randomus online random number generator (https://randomus.ru).

The number of respondents exceeded the number required for pilot studies, with a significance level of $p = 0.05$ [15]. Among the respondents, there were 25 (53.2%) men and 22 (46.8%) women. The average age of the respondents was 32.3 ± 4.94 years; the difference in age between men (33.7 ± 5.32 years) and women (30.8 ± 3.94 years) was significant ($t = 3.69$; $p = 0.011$). Among 47 respondents, 24 (51.1%) were surgeons, including dentists, and 23 (48.9%) were general practitioners, including dentists. There was no difference in the age of respondents with surgical (32.5 ± 5.36 years) and therapeutic (32.1 ± 4.47 years) specialties ($t = 0.49$; $p = 0.388$). Among the respondents, 25 (53.2%) worked in medical organizations providing outpatient care, and 22 (46.8%) worked in hospitals. There was no difference in age between the respondents of these two groups: 31.8 ± 4.44 years and 32.9 ± 5.42, respectively ($t = 1.39$; $p = 0.316$). The respondents' distribution by selected characteristics is presented in Table 2.

The survey was conducted using a questionnaire developed by the Department of Economics and Marketing at the Postgraduate Medical Education Academy. The questionnaire contained two questions about the sufficiency and clarity of the website information about the rules and procedures for submitting documents for certification, as follows:

1. How complete is the information provided to answer your questions about the rules and procedures for submitting documents for certification?

2. To what extent is the information presented clear to you, and does it require additional explanation or search for information on other resources?

To formalize the respondents' opinions on the sufficiency and clarity of the information about the rules and procedures for submitting documents for certification, a five-point rating scale was used, where "1" represents an extreme lack of information and "5" represents an absolute sufficiency and clarity of the presented information.

Statistical processing was performed using the SPSS software (IBM Company). The availability of information about the list of certification specialties, the rules and procedures for submitting documents, and sample tests was evaluated using frequency analysis. The analysis of respondents' opinions about the sufficiency and clarity of information about the rules and procedures for

Table 1. Criteria for evaluating the information content of websites of territorial certification commissions

Criteria	Presence (yes/no)
List of specialties	
Personal composition of the attestation commission	
Personal composition of the expert groups	
Information in paragraph 34.1 of the Order	
Information in paragraph 34.2 of the Order	
Information in paragraph 34.3 of the Order	
Information in paragraph 34.4 of the Order	
Information in paragraph 34.5 of the Order	
Information in paragraph 34.6 of the Order	
Information in paragraph 34.7 of the Order	
Information in paragraph 34.8 of the Order	
Information in paragraph 37 of the Order	
Information in paragraph 39 of the Order	
Information in paragraph 35 of the Order	
Information in paragraph 36 of the Order	
AC mailing address	
AC email	
Information about the possibility of submitting documents on the Public Services Portal of the Russian Federation	
Document acceptance schedule	
Current document registration schedule	
Current dates of the attestation commissions meetings	
Current dates of expert commission meetings	
Time and place of certification for on-site meetings	
Current dates of the test control	
Current interview dates	
Order Link	

Table prepared by the authors

Note: AC — attestation commission.

Table 2. Respondent distribution

Medical specialty	Sex	Medical facilities		Total
		in-patient	out-patient	
Surgery	m	11	1	12
	f	11	1	12
	total	22	2	24
Internal medicine	m	–	13	13
	f	–	10	10
	total	–	23	23
Total	m	11	14	25
	f	11	11	22
	total	22	25	47

Table prepared by the authors

Note: – not available.

submitting documents for certification was conducted using frequency analysis and the calculation of average values (mean, standard deviation, and median). Since the variables "sufficiency" and "clarity" did not have a normal distribution ($p \leq 0.001$), the Mann–Whitney and Kruskal–Wallis criteria and Spearman's rank correlation coefficient were used.

RESULTS

The conducted search revealed that in 17 RF subjects, the territorial attestation commissions have separate websites, including those without a link on the websites of the authority organ. The analysis showed that most websites of territorial attestation commissions contain the basic information required by the Order[3] (Table 3). However, only 47 websites (56.6%) contained information on all Order paragraphs regarding the rules and procedures for submitting documents. Only in 14 (16.9%) RF subjects, the websites of healthcare executive authorities and territorial attestation commissions simultaneously provide information on the rules and procedures for submitting documents, the list of attestation specialties, attestation test samples, and reference to the current Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023)[4], as well as information on the current dates of meetings of attestation/expert commissions.

Out of 18 websites that featured sample tests, 5 (6.1%) websites provided a link to the Central Commission for the Certification of Medical Workers for the Assignment of Qualification Categories. Only 21 (25.3%) websites had an active link to the current order of the Ministry of Health of the Russian Federation, and 13 (15.7%) websites mentioned that the procedure for medical and pharmaceutical workers to pass certification for obtaining a qualification category was regulated by the current order. 34 (41.0%) websites did not contain information about the current regulatory legal act, and 3 websites contained an invalid order issued by the Ministry of Health of the Russian Federation on April 23, 2013, No. 240N.

According to the survey results, the average score given by the respondents for the sufficiency of information about certification of medical professionals on 47 websites was 3.13 ± 1.04 (median 3.0). Concerning the content clarity, the score was 2.98 ± 1.02 (median 3.0).

Regarding the sufficiency of information, the respondents gave the highest score of 4.33 to 5 (10.6%) websites, and the lowest score of 1.33 to 4 (8.5%) websites. According to the respondents, 2 (4.3%) websites received the highest score of 4.68 for the clarity of information, while 1 (2.1%) website received the lowest score of 1.0.

According to the respondents, the clarity of information about the certification of medical professionals is worse than its sufficiency ($p = 0.009$). The opinions of the respondents about the sufficiency and clarity of information on different websites differ significantly: $102.155 \leq \chi^2 \leq 110.978$ ($p \leq 0.001$). When comparing the opinions of respondents on the same websites, all

[3] Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023) "On Approval of the Procedure and Terms for Medical and Pharmaceutical Professionals to Obtain a Qualification Grade".

[4] Ibid.

Table 3. Analysis of the websites of territorial attestation commissions on the availability of information on the procedure and deadlines for medical and pharmaceutical professional to obtain a qualification grade

Information in accordance with the Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023)	Number of websites with information available
List of specialties	64 (77.1%)
Personal composition of attestation commission	66 (79.5%)
Personal composition of expert groups	49 (59.1%)
Rules for submitting documents	
Information in paragraph 34.1 of the Order	74 (89.2%)
Information in paragraph 34.2 of the Order	78 (93.9%)
Information in paragraph 34.3 of the Order	66 (79.5%)
Information in paragraph 34.4 of the Order	76 (91.6%)
Information in paragraph 34.5 of the Order	78 (93.9%)
Information in paragraph 34.6 of the Order	66 (79.5%)
Information in paragraph 34.7 of the Order	52 (62.7%)
Information in paragraph 34.8 of the Order	49 (59.1%)
Information in paragraph 37 of the Order	53 (63.9%)
Information in paragraph 39 of the Order	51 (61.5%)
Procedure for submitting documents	
Information in paragraph 35 of the Order	77 (92.8%)
Information in paragraph 36 of the Order	67 (80.7%)
AC mailing address	76 (91.6%)
AC email	69 (83.1%)
Information about the possibility of submitting documents on the Public Services Portal of the Russian Federation	54 (65.1%)
Document acceptance schedule	78 (93.7%)
Current document registration schedule	51 (61.5%)
Current dates of the attestation commissions meetings	35 (42.2%)
Current dates of expert commission meetings	35 (42.2%)
Time and place of certification for on-site meetings	18 (38.3%)
Current dates of the test control	18 (38.3%)
Current interview dates	41 (87.2%)
Attestation test samples	18 (21.7%)
Link to the Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023)[5]	36 (43.4%)

Table prepared by the authors

[5] Ibid.

respondents gave the same rating for the sufficiency (p = 0.881) and clarity (p = 0.976) of the information presented.

The respondents' opinions did not depend on their age when evaluating the websites based on information sufficiency (p = 0.416) and content clarity (p = 0.706); no statistically significant differences were found during the correlation analysis.

The opinions of respondents of different genders about the sufficiency (p = 0.163) and clarity (p = 0.148) of the information were identical. The opinions of respondents of surgical and therapeutic specialties about the sufficiency (p = 0.901) and clarity (0.947) of the content were also identical. The opinions of respondents working in hospitals and medical organizations providing outpatient care about the sufficiency (p = 0.956) and clarity (0.983) of the websites were also identical.

DISCUSSION

According to the results obtained, out of 83 RF subjects, only 14 (16.9%) have the required information about the rules and procedures for submitting documents, the list of attestation specialties, attestation test samples, and active links to the current Order of the Ministry of Health of the Russian Federation (No. 458n dated August 31, 2023), as well as information about the current dates of the attestation/expert commission meetings.

Information on all Order paragraphs regarding the rules and procedure for submitting documents is presented on 47 (56.6%) websites. When evaluating these websites, the respondents considered the sufficiency of information on the rules and procedure for submitting documents to be satisfactory, while the content clarity was significantly lower than satisfactory. Thus, even at the stage of preparing for attestation for a qualification grade, the applicant will still have questions about the rules and procedure for submitting documents, requiring additional explanations and/or search for information on other resources.

It should be noted that the consistency of the respondents' opinions about the sufficiency and clarity of information on the same websites indicates their impartiality. The lack of correlation between the respondents' opinions and their age, gender, specialty, or the type of medical facility eliminates the influence of sociodemographic factors. Given that the average age of the respondents was 32 years, we cannot assume that they lack competencies in using online resources.

The discrepancy between the respondents' assessments of the sufficiency and clarity of information on different websites indirectly reflects the attitude of the attestation commissions towards the quality of the information content on their websites. In our opinion, the poor presentation, delayed updates, low clarity, and insufficient information about certification procedures demonstrate the indifference of some regional healthcare executive authorities towards the institution of medical qualification.

Given that the number of respondents corresponds to that required for a pilot study, the opinion on the sufficiency and clarity of the information about certification of medical professionals needs to be confirmed by surveying a larger number of specialists.

The survey was conducted by medical professionals who have good skills of online information search, which may distort the results in favor of higher ratings. Despite the survey being conducted remotely, it is possible that the respondents who were currently studying at the same organization might discuss the subject of the survey. Additionally, there may be a bias towards positive or negative attitudes towards the institution of qualification categories.

CONCLUSION

The information about the attestation procedure of medical professionals, which is available on the websites of the healthcare executive authorities of the Russian Federation subjects and on the websites of the respective territorial attestation commissions, needs to be corrected and updated.

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