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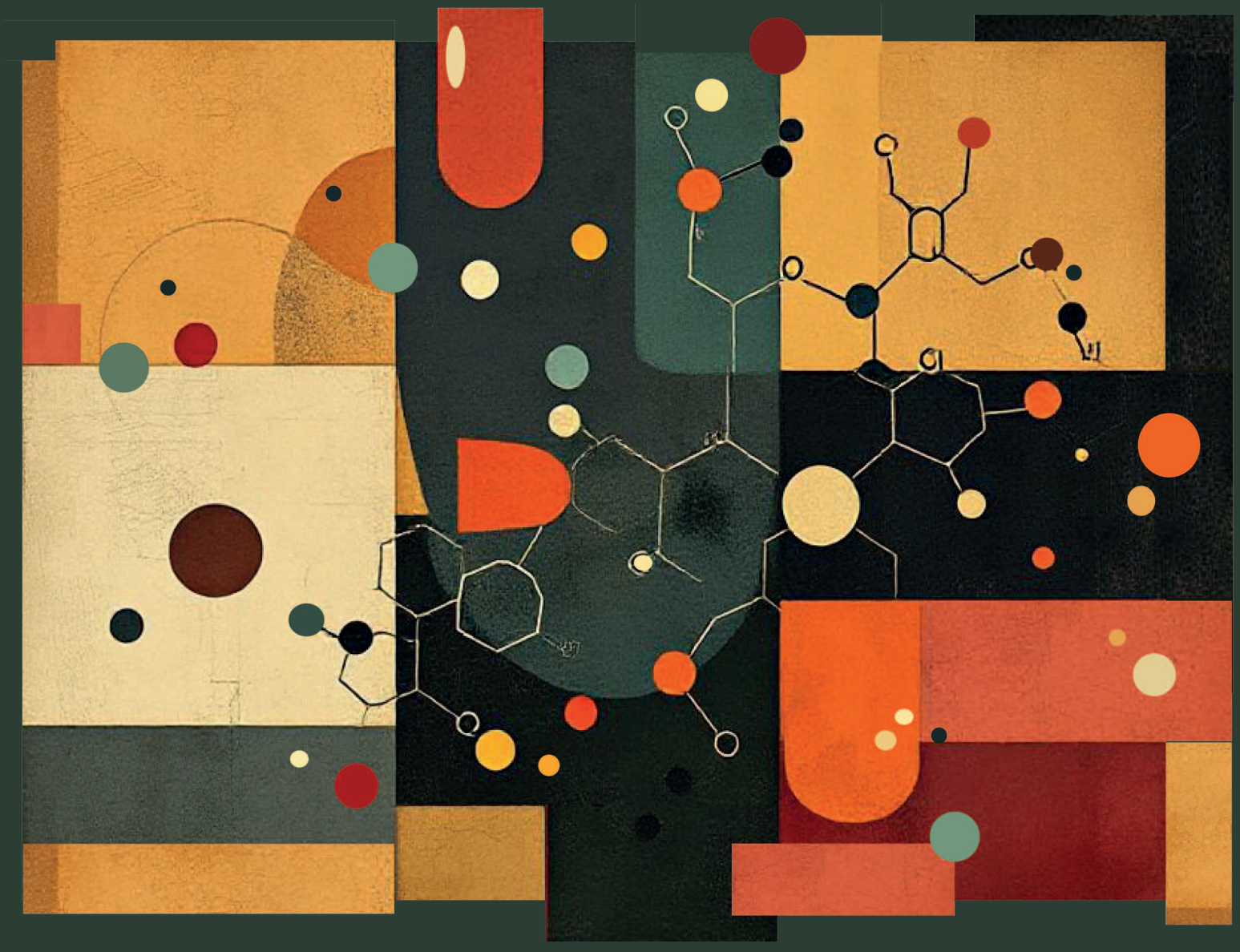

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ТОМ 27, № 4, 2025

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EVALUATION OF ACUTE TOXICITY AND PHARMACOKINETICS OF A NATURAL PHAEOSPHAERIDE A DERIVATIVE IN LABORATORY RODENTS

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Introduction. The determination of metabolism and pharmacokinetics is an essential requirement in the development of any drug. Phaeosphaeride A (PPA) is an anticancer agent belonging to the group of natural compounds with antitumor properties, which was first isolated from the endophytic fungus FA39 by Harvard scientists (Claudy et al.) in 2006. In this study, we investigate compound AV6, which is a derivative of natural phaeosphaeride A.

Objective. To study the acute toxicity and pharmacokinetic characteristics of the semi-synthetic substance AV6 obtained based on phaeosphaeride A, a natural phytotoxin with antitumor properties, following a single intragastric administration of AV6 in laboratory rodents.

Materials and methods. The acute toxicity of AV6 was studied using 30 male Balb/c mice, which were divided into five groups of six animals each. The control group received a single intragastric administration of a solvent (oil-alcohol emulsion, 300 μL volume), while four experimental groups received AV6 at doses of 5, 50, 300, and 2000 mg/kg bw. Body weight dynamics were evaluated, and organ mass coefficients were calculated. The pharmacokinetic study was performed following a single intragastric administration of AV6 at a dose of 25 mg/kg bw to outbred male Wistar rats. The AV6 dose for the pharmacokinetic study was determined based on acute toxicity data, accounting for the interspecies conversion factor. Quantitative determination of AV6 in blood plasma and urine was performed using the MS/MS method. Statistical analysis was conducted using GraphPad Prism 5 software.

Results. According to the acute toxicity data following intragastric administration, the AV6 phaeosphaeride A derivative can be classified as hazard class 3 (animal mortality was observed exclusively in the 2000 mg/kg bw group). Visual examination of internal organs revealed no apparent macroscopic signs of pathology. No statistically significant changes in the mass coefficients of internal organs were detected in experimental animals compared to controls. A quantitative determination procedure for AV6 was developed based on HPLC–MS/MS analysis. Metabolites formed in rats *in vivo* were identified. A comparison of rat blood plasma chromatograms 1 h and 10 h after intragastric AV6 administration showed that, after 1 h, the AV6 peak intensity was 20 times higher than the M2 peak. However, after 10 h, the AV6 peak intensity decreased, while the metabolite M2 peak intensity increased.

Conclusion. Compound AV6 is classified as a moderately hazardous substance. Data on the structure of AV6 metabolites (a derivative of natural phaeosphaeride A) obtained during pharmacokinetic studies in rats indicate a relatively low metabolic rate of the compound. This is primarily due to chemical transformations at the nitrogen atom of the lactam ring, resulting in metabolites that may be excreted in urine. The most probable mechanisms of these transformations are oxidative deacylation followed by hydrolysis. The completed preclinical study evaluating the acute toxicity, metabolism, and pharmacokinetics of AV6 represents a crucial step in translating previous findings on the antitumor potential of this derivative of natural phaeosphaeride A and advancing *in vivo* research.

Keywords: natural phaeosphaeride A; pharmacokinetics; metabolism; acute toxicity; HPLC–MS analysis

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ОЦЕНКА ОСТРОЙ ТОКСИЧНОСТИ И ФАРМАКОКИНЕТИКИ ПРОИЗВОДНОГО ПРИРОДНОГО ФЕОСФЕРИДА А НА ЛАБОРАТОРНЫХ ГРЫЗУНАХ

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Введение. Определение метаболизма и фармакокинетики является важным условием при разработке любого лекарственного препарата, в том числе противоракового феосферида А (PPA), который относится к группе природных соединений, обладающих

противоопухолевыми свойствами, и был впервые выделен из эндофитного гриба FA39 гарвардскими учеными (Клауди и его коллегами) в 2006 году. Объектом исследования выбрано соединение AV6 — производное природного феосферида А.

Цель. Изучение острой токсичности и особенностей фармакокинетики полусинтетической субстанции AV6 на платформе природного фитотоксина феосферида А, обладающего противоопухолевыми свойствами, при однократном внутрижелудочном введении соединения AV6 на лабораторных грызунах.

Материалы и методы. Исследование острой токсичности AV6 выполнено на самцах мышей Balb/c (30 самцов были разделены на 5 групп по 6 животных в каждой группе). Контрольной группе однократно внутрижелудочно вводили растворитель (масляно-спиртовую эмульсию в объеме 300 мкл), четыре группы (экспериментальные животные) получали AV6 в дозах 5, 50, 300 и 2000 мг/кг м.т. Оценивали динамику массы тела животных, рассчитывали массовые коэффициенты органов. Исследования фармакокинетики выполнены при однократном внутрижелудочном введении AV6 в дозе 25 мг/кг м.т. аутбредным крысам-самцам Wistar. Доза AV6 для фармакокинетического исследования рассчитана на основании данных об острой токсичности с учетом коэффициента межвидового переноса. Для количественного определения AV6 в плазме крови и моче использован МС/МС метод. Статистический анализ проведен с использованием ПО GraphPad Prism 5.

Результаты. На основании данных об острой токсичности при внутрижелудочном введении производное феосферида А — AV6 можно отнести к 3-му классу опасности (гибель животных наблюдали исключительно в группе 2000 мг/кг м.т.). При визуальной оценке внутренних органов явных макроскопических признаков патологии выявлено не было. Также не было обнаружено статистически значимых изменений массовых коэффициентов внутренних органов экспериментальных животных по сравнению с контролем. Разработана процедура количественного определения AV6 на основе ВЭЖХ-МС/МС анализа. Определены метаболиты, образующиеся в организме крыс *in vivo*. При сравнении хроматограмм плазмы крови крысы через 1 час после внутрижелудочного введения AV6 и спустя 10 часов после введения установлено, что через час после введения пик AV6 по интенсивности в 20 раз превосходил пик M2. Однако через 10 часов интенсивность пика AV6 уменьшилась, в то время как интенсивность пика метаболита M2 увеличилась.

Выводы. Соединение AV6 относится к умеренно опасным веществам. Данные о структуре метаболитов AV6, производного природного феосферида А, полученные в ходе фармакокинетического исследования на крысах, свидетельствуют о невысокой скорости метаболизма вещества, что обусловлено преимущественно химическими превращениями у атома азота лактамного цикла, в результате чего образуются метаболиты, которые могут выделяться в составе мочи. Наиболее вероятными механизмами таких превращений являются окислительное деацилирование и следующий за ним гидролиз. Выполненное доклиническое исследование по оценке острой токсичности AV6, его метаболизма и фармакокинетики является одним из этапов для переноса полученных ранее авторами данных о противоопухолевом потенциале этого производного природного феосферида А и дальнейшей реализации исследований *in vivo*.

Ключевые слова: природный феосферид А; фармакокинетика; метаболизм; острая токсичность; ВЭЖХ-МС анализ

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INTRODUCTION

Cancer, along with cardiovascular diseases, continues to be a leading cause of mortality worldwide. According to WHO projections, cancer incidence will increase by 70% over the next 20 years, becoming the cause of nearly one in six deaths globally [1].

For the past 50 years, chemotherapy has remained the primary treatment for cancer, despite its serious side effects. Among them are multidrug resistance, hepatotoxicity (which was until recently considered an inevitable consequence of chemotherapy), and other significant adverse effects[1] [2]. The underlying causes of multiple adverse drug reactions fall within the research domains of molecular biology, cytology, genetics, and related disciplines.

The development prospects of any anticancer drug depend on understanding its molecular mechanisms of action, specific tumor-targeting effects and influence on the tumor microenvironment, pharmacokinetic properties, toxicity and safety profile, as well as other critical factors.

Achievement of therapeutic *in vivo* concentrations sufficient for full clinical efficacy represents a major challenge in anticancer drug development[2]. Essential data for drug development must also include information on the systemic effects of potential anticancer compounds in animal models used for toxicological evaluation of drug prototypes, enabling dose extrapolation to humans[3].

[1] Luellman H, Mohr K, Hein L. Pocket atlas of pharmacology. 4th ed. Thieme; 2011.
[2] Key Scientific Achievements in Medicinal Chemistry in 2023. Scientific Council on Medicinal Chemistry of the Russian Academy of Sciences, Division of Chemistry and Materials Science of the Russian Academy of Sciences. 2024.
[3] Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2) CPMP/ICH/286/95 (2009). The European Agency for the Evaluation of Medicinal Products. Human medicines evaluation unit.

Preclinical drug development requires comprehensive pharmacokinetic studies in animal models to evaluate optimal efficacy and safety profiles. Historically, such studies primarily utilized radiolabeled compounds. Over the past two decades, analytical approaches based on tandem mass spectrometry (MS/MS) have become widely adopted, proving effective for both clinical therapeutic drug monitoring and preclinical development of novel pharmaceuticals [3].

In our previous research, we investigated AV6, a derivative of natural phaeosphaeride A [4] with the chemical structure (2S,3R,4R)-3-hydroxy-6-methoxy-3-methyl-7-methylene-2-pentyl-4-pyrrolidin-1-yl-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2H)-one [5-10], and established its:

- *in vitro* cytotoxicity against various tumor cell lines;
- inhibitory activity against P-glycoprotein (MDR1), the primary xenobiotic transporter.

These dual properties — intrinsic antitumor activity and ability to reduce MDR1 substrate efflux — render AV6 a promising lead compound for anticancer drug development.

In this study, we aim to investigate the acute toxicity and pharmacokinetic properties of semi-synthetic AV6, based on the natural phytotoxin phaeosphaeride A with established antitumor activity, following single intragastric administration in laboratory rodents.

MATERIALS AND METHODS

The study was conducted in compliance with the requirements of Federal Law No. 61-FZ dated 12.04.2010 (Russian Federation)[4], the national standard GOST R 53434-2009[5], Good Laboratory Practice (GLP) principles[6], Guidelines on preclinical research[7]. The requirements for the housing and care of animals used for scientific purposes comply with Directive 2010/63/EU[8].

The acute toxicity of AV6 was evaluated in male Balb/c mice (8–10 weeks old, body weight 17.5–19.5 g) obtained from the Rappolovo Laboratory Animal Breeding Facility.

The acute toxicity study of AV6 in mice was performed to establish those dose levels that do not cause acute intoxication or animal death following single intragastric administration of an oil-alcohol emulsion of the substance. The selection of this administration form for AV6 was based on the need to obtain a homogeneous mixture and to enhance the bioavailability of the hydrophobic AV6 compound, since dilution of initial AV6 solutions in other solvents followed by water dilution resulted in immediate precipitation.

Experiments on acute toxicity evaluation via intragastric administration with determination of the test substance hazard class were conducted in accordance with GOST 32644-2014, identical to the international document OECD Test Guideline No. 423:2001 (Acute Oral Toxicity — Acute Toxic Class Method, IDT).

The AV6 emulsion for intragastric administration was prepared by dissolving a weighed portion of the substance in a minimal volume of ethanol (10–72 μL, depending on the required amount of AV6) followed by addition of an oil-alcohol mixture such that the administration volume always amounted to 300 μL. The final concentration of AV6 in the emulsion depended on the animal's body weight and the dose used in the experiment, with ethanol concentration in the preparation being ~23%. The addition of oil to the alcohol solution of AV6 allowed a homogeneous mixture to be obtained, thus ensuring uniform dosing conditions for the study purposes. Food was withdrawn 2 h before drug administration and access to food was restored 2 h after administration. Intragastric administration was performed using a mouse gastric tube and syringe.

All treated animals (30 males) were divided into five groups of six animals each. The control group received intragastric administration of the solvent (oil-alcohol emulsion in a volume of 300 μL), while four groups (experimental animals) received AV6 at doses of 5, 50, 300, and 2000 mg/kg bw.

Observation of the animals was carried out for the first 30 min after dose administration, then periodically during the first 24 h and daily for a total of 14 days to record any clinical signs of toxicity or mortality. The body weight of the animals was measured one day after administration and at the end of each week of observation. Upon completion of the experiment after 14 days, the general toxic effect of AV6 was evaluated. For this purpose, blood samples were collected from all surviving animals, which were then subjected to cervical dislocation, and internal organs — the heart, lungs, liver, kidneys, spleen, and thymus — were extracted to assess their appearance and determine their weights for calculating organ-to-body weight ratios (the ratio was calculated as organ weight (g)/body weight (g) × 100).

The pharmacokinetic evaluation of AV6 was conducted on five outbred male Wistar rats weighing 350–380 g from the Rappolovo Laboratory Animal Breeding Facility.

All animals underwent quarantine and acclimatization to the animal facility conditions before the onset of the experiment. The rodents were housed in groups of five per cage with free access to food and water. A complete granulated feed "CHARA" for laboratory rodents was used as the diet. The animal facilities maintained a day/night cycle (12 h/12 h), a temperature of 20–26°C, and humidity of 30–70%.

[4] Federal Law No. 61-FZ of 12.04.2010 "On Circulation of Medicinal Products".
[5] GOST R 53434-2009 National Standard of the Russian Federation Principles of Good Laboratory Practice (GLP) Moscow: Standartinform; 2010.
[6] Good laboratory practice: OECD principles and guidance for compliance monitoring. 2005.
[7] Mironov A.N., editor. Guidelines for Conducting Preclinical Studies of Pharmaceuticals. Part One. Moscow: Grif & K; 2012.
[8] European Parliament and Council. Directive 2010/63/EU of the European Parliament and of the council of 22 Sept. 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union. 2010; 276:33–79.

Catheterization of the jugular vein in rats used in pharmacokinetic studies was performed under inhalation anesthesia with a gas mixture (5% isoflurane in air) using an Univentor 410 Anaesthesia Unit (Univentor Ltd, Malta).

The animals were placed in a supine position, and their front and hind limbs were secured with adhesive tape to the surface of a heated and illuminated surgical table. After achieving surgical access to the *v. jugularis externa* and placing a cranial ligature around the vessel, catheterization was performed. The catheter was inserted into the vein toward the heart until the catheter ring reached the vessel incision site. In this position, the catheter tip was sufficiently close to the entrance of the right atrium.

The catheter was then secured in the vessel using the remaining ends of the previously placed cranial ligature. After fixation, the catheter was tunneled subcutaneously to the scruff of the neck and then exteriorized dorsally between the scapulae through a small incision (~1 cm). Following wound closure, a harness was placed on the animal, through which the free end of the catheter was routed for connection to an Accusampler automatic blood sampling device.

Under these conditions, the rat was housed in a specialized cage with minimal movement restrictions. Throughout the post-catheterization period, the catheter was automatically flushed every 30 min with heparinized saline (16 IU/mL) in a volume of 20 µL.

Before initiating pharmacokinetic studies, at least 36 h were allowed after surgery to ensure animal recovery and minimize the influence of surgical trauma and anesthetic agents on the absorption, metabolism, and elimination of the test compound.

Pharmacokinetic studies

The pharmacokinetic studies were conducted following a single intragastric administration of a guaranteed safe dose of AV6 (25 mg/kg bw) in an oil-alcohol emulsion, which was selected based on the results of acute toxicity studies in mice, taking into account known interspecies conversion factors for rats.

Prior to drug administration, baseline blood samples of 0.2 mL were collected from all rats. The Accusampler system used for *in vivo* blood sampling ensured periodic flushing of the device's capillaries and valves with heparinized saline (16 IU/mL) and allowed for the collection of whole blood in predetermined 200 µL volumes without dilution. After each blood sample collection, the volume was automatically replaced with heparinized saline. Automated blood sampling was performed at 20, 30 min, 1, 2, 4, 6, 8, 24, and 48 h. The sample tubes were centrifuged (5 min, 11,000 rpm) at room temperature. The supernatant (approximately 100 µL) was transferred using a mechanical pipette into Eppendorf tubes, frozen, and stored for subsequent analysis at minus 80°C for no more than two months.

Urine samples (0.3–0.5 mL) were collected at intervals before drug administration, as well as at 8, 24, and 48 h after administration. The collected samples were centrifuged (3 min, 14,000 rpm) to remove insoluble precipitates. The supernatant was transferred using a mechanical pipette into Eppendorf tubes, frozen, and stored at minus 80°C for no more than 2 months. Centrifugation was performed using a Hermle Z160M centrifuge with an angular rotor (rotor radius — 8.5 cm).

In the study of the pharmacokinetic characteristics of AV6 and its potential metabolites, the following parameters were determined: elimination rate constant (k_e), half-life ($T_{½}$), area under the pharmacokinetic curve ($AUC_{0\rightarrow48}$), volume of distribution (*V*d), and total clearance during the elimination phase (CLtot).

Sample preparation of urine and blood plasma for AV6 and metabolites determination

In 2.0 mL Eppendorf tubes, 0.05 mL of the analyzed sample (blood plasma or urine) was added, followed by 0.15 mL of acetonitrile. The mixture was thoroughly vortexed (BioSan FVL-2400n) and centrifuged for 3 min at 14,000 rpm. The supernatant was transferred into a 0.2 mL insert chromatographic vial. The vials were placed into the chromatograph autosampler, with an injection volume of 0.005 mL.

Quantification of AV6 and its metabolites was performed using an UltiMate 3000 liquid chromatograph equipped with an autosampler and a Q-Exactive mass-selective detector with electrospray ionization at atmospheric pressure. Data acquisition and processing were performed using the Xcalibur software.

For quantitative determination and precise confirmation of the presence of AV6 in blood plasma or urine, an MS/MS method was employed. The confirmation of AV6/metabolites in complex matrices (blood plasma or urine) was based on the detection of three product ions characteristic of the analyzed substance. Given the preliminary nature of the study, which primarily aimed to identify AV6 and potential metabolites, the complete validation method for quantitative determination was not conducted. The AV6 reference material used for calibration samples was preliminarily analyzed by NMR (Bruker AVANCE III 400 MHz NMR spectrometer); the purity of at least 99% was confirmed.

For calibration sample preparation, 0.495 mL of blood plasma or urine and 0.005 mL of AV6 solution (1 mg/mL in methanol) were dispensed into a 2.0 mL Eppendorf tube. The resulting blood plasma or urine samples contained AV6 at a concentration of 10 µg/mL. Serial dilutions (by adding 0.05 mL of the prepared AV6 solution to 0.45 mL of blood plasma or urine) yielded solutions with AV6 concentrations of 1, 0.1, and 0.01 µg/mL.

For analysis, 0.05 mL of each calibration sample (blood plasma or urine) was dispensed into a 2.0 mL Eppendorf tube, followed by 0.15 mL of acetonitrile. The mixture was thoroughly vortexed and centrifuged (3 min, 14,000 rpm). The supernatant was transferred into a 0.2 mL insert chromatographic vial, which was then placed into the chromatograph autosampler. The injection volume was 0.005 mL.

Chromatographic separation conditions

Column Specifications: column Zorbax SB-C8 (Agilent), length 150 mm, internal diameter 4.6 mm, sorbent particle size 1.8 μm. Mobile Phase Composition:

- Component A: 0.1% formic acid solution in deionized water
- Component B: 0.1% formic acid solution in gradient-grade HPLC acetonitrile

Chromatographic Elution Mode: Gradient elution. Mobile phase component ratios are shown in Table 1.

The study was conducted with the following chromatographic parameters: a flow rate of 0.4 mL/min, a column oven temperature of 35°C, an injection volume of 5.0 μL, the total run time of 14 min.

Mass spectrometric detection parameters

The study was performed under the following operating conditions of the Q-Exactive mass spectrometric detector with electrospray ionization at atmospheric pressure: drying gas flow — 45 arbitrary units; auxiliary gas flow — 20 arbitrary units; sprayer pressure — 35 psi; drying gas temperature — 350°C; auxiliary gas stream temperature — 400°C; capillary voltage — 3500 V. Detection was carried out in full ion current scanning mode (SCAN), with ion registration in the m/z range 60–900 amu throughout the entire analysis (under positive ionization).

Ionization source mode: electrospray ionization at atmospheric pressure.

Detection mode: detection under positive ionization in MS and MS/MS modes at high resolution.

Detected ion polarity: detection of positive ions.

Scanning mode: detected mass range, m/z 60–950 amu, collision cell voltage — 25 arbitrary units.

The high-resolution mass spectrometry (HRMS) mode was used to acquire mass chromatograms based on the exact masses of the molecular ions of the compounds listed above. Statistical analysis was performed using the GraphPad Prism 5 software.

RESULTS AND DISCUSSION

Acute toxicity of AV6 upon intragastric administration

During the experiment, animal mortality was observed exclusively in the 2000 mg/kg bw group (three mice (50%) died on the second day after AV6 administration). On this basis, the studied phaeosphaeride A derivative (AV6) can be classified as hazard class 3, since the LD_{50} value for AV6 in the form of an oil-alcohol emulsion upon intragastric administration, according to the obtained data, is close to the maximum tested dose of 2000 mg/kg bw.

Measurements of animal body weight in the experimental groups showed its low dependence on the effects of AV6 upon intragastric administration. The results of mouse body weight measurements and organ-to-body weight ratios are presented in Tables 3 and 4.

Visual examination of internal organs revealed no apparent macroscopic pathological alterations. Furthermore, the comparative analysis demonstrated no statistically significant differences in organ-to-body weight ratios between experimental and control animal groups.

Pharmacokinetic study of AV6 and identification of its metabolites

The pharmacokinetics of AV6 and identification of its potential metabolites were investigated following single intragastric administration of AV6 (25 mg/kg bw). The

Table 1. Mobile phase composition gradient profile

Time, min	Component, %	
	A	B
0.00	60	40
0.50	60	40
7.00	10	90
10.00	10	90
10.10	60	40
14.00	60	40

Table prepared by the authors using their own data

Table 2. Fragmentation conditions for AV6

Mass [m/z]	Formula [M]	Species	Polarity	(N)CE	(N)CE type	Comment
351,22783	$C_{19}H_{30}N_2O_4$	+ H	Positive	25	NCE	AV-6

Table prepared by the authors using their own data

[10] Test Guideline 423. OECD guideline for testing of chemicals. Acute oral toxicity — Acute toxic class method. 2002b. OECD, Sep. 2021.

Table 3. Results of animal body weight assessment (g)

Observation period	Animal groups				
	Control *n* = 6	5 mg/kg *n* = 6	50 mg/kg *n* = 6	300 mg/kg *n* = 6	2000 mg/kg*
Day 1	18.7 ± 0.7	20.0 ± 0.3	18.1 ± 0.6	19.0 ± 0.7	18.3 ± 0.7
Week 1	18.9 ± 0.8	20.1 ± 0.1	19.3 ± 0.4	19.4 ± 0.9	18.4 ± 1.6
Week 2	19.2 ± 0.8	20.4 ± 0.4	19.0 ± 0.5	19.6 ± 1.0	19.1 ± 1.4

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Note: * — the presented data are for surviving animals only (*n* = 3); the data are presented as mean ± standard error of the mean (*M* ± *m*).

Table 4. Organ-to-body weight ratios (relative organ weights)

Organ	Animal groups				
	Control *n* = 6	5 mg/kg *n* = 6	50 mg/kg *n* = 6	300 mg/kg *n* = 6	2000 mg/kg *n* = 3
Heart	0.54 ± 0.06	0.50 ± 0.03	0.57 ± 0.06	0.53 ± 0.08	0.58 ± 0.04
Liver	5.16 ± 0.33	5.05 ± 0.30	4.86 ± 0.16	4.86 ± 0.36	5.57 ± 0.15
Kidneys	0.71 ± 0.07	0.70 ± 0.04	0.66 ± 0.05	0.68 ± 0.05	0.76 ± 0.07
Spleen	0.53 ± 0.10	0.59 ± 0.12	0.49 ± 0.03	0.47 ± 0.03	0.54 ± 0.20
Lungs	1.12 ± 0.32	0.88 ± 0.10	1.00 ± 0.13	0.91 ± 0.15	1.10 ± 0.25
Thymus	0.25 ± 0.06	0.24 ± 0.09	0.25 ± 0.07	0.23 ± 0.06	0.32 ± 0.06

Table prepared by the authors using their own data

Note: the data are presented as mean ± standard error of the mean (*M* ± *m*).

concentration-time data for AV6 were analyzed using a non-compartmental approach.

The absorption pharmacokinetics of AV6 after intragastric administration was characterized by a relatively rapid achievement of maximum concentration (30 ng/mL), with T_{max} ranging 30–60 min. The formal conversion based on rat blood volume (58–64 mL/kg bw) [11, 12] suggests that approximately 10–15% of the intragastrically administered AV6 dose was absorbed into the systemic circulation of experimental rats.

Table 5. Pharmacokinetic parameters in rats following single intragastric administration of AV6 at 25 mg/kg bw

Parameter	Parameter Value
Elimination rate constant (k_e), h^{-1}	0.138–0.219
Half-life ($T_{½}$), h	3.2–5.0
Area under the (pharmacokinetic) concentration-time curve ($AUC_{0\rightarrow48}$), μg×h/L	80–98
Volume of distribution (*V*d), L	~50
Total clearance during elimination phase (CLtot), mL/min	~165

Table prepared by the authors using their own data

The elimination phase of AV6 concentration showed exponential decay. The elimination rate constant (k_e) was determined from the slope of the concentration–time curve in semi-logarithmic coordinates using data from five animals.

The area under the concentration–time curve (AUC) was calculated using blood concentration data from 20 min to 48 h post-administration, applying the trapezoidal rule. The volume of distribution (*V*d) was estimated considering the assumed oral bioavailability (approximately 15%) of AV6. The pharmacokinetic parameters following intragastric administration of AV6 are presented in Table 5.

The exponential approximation generally provides adequate description of AV6 concentration–time curves during the elimination phase. However, certain experiments demonstrated an increase in plasma concentration within 2–5 h post-administration. This kinetic pattern most likely reflects substance redistribution from circulation into hydrophobic compartments (intercellular

spaces) with subsequent re-entry into systemic circulation. Potential plasma protein binding effects on AV6 extraction from biological samples should also be considered.

The elimination phase shows rapid AV6 concentration decline, as evidenced by the determined k_e and T½ values. This kinetic behavior presumably results from metabolic transformation of the compound, supported by detection of significant M2 metabolite levels in plasma 10 h post-dosing.

Urinary excretion assessment of AV6 and its metabolites was based on concentration measurements in urine samples collected at intervals over 48 h following intragastric administration. Cumulative 24-h excretion (normalized to approximate daily urine volume of ~12 mL) ranged 750–1400 ng, representing 50–93% of systemically absorbed drug (assuming 15% oral bioavailability).

At this stage of the study, data on two major metabolites were obtained. Figures 2–4 present chromatograms of blood plasma samples from experimental animals. In addition to the parent compound, the samples demonstrated the presence of its metabolites M1 and M2.

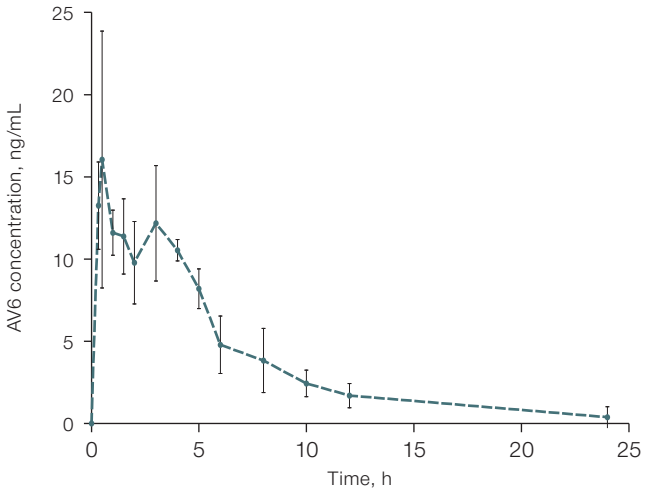

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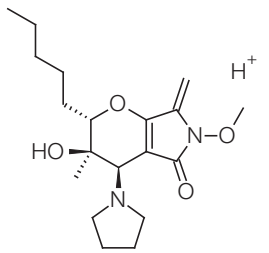
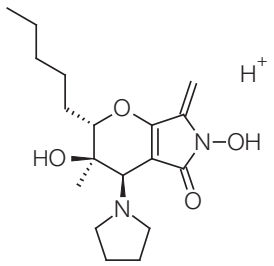
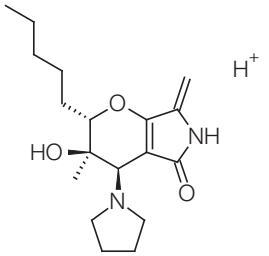
Fig. 1. Plasma concentration-time profile of AV6 in rats following single intragastric administration

Note: the graph was generated using the GraphPad Prism 5 software; the data are presented as mean ± standard error of the mean ($M \pm \delta$).

Table 6. Molecular ion peaks of AV6 and its metabolites (M1 и M2)

Code	Gross formula	Molecular weight	m/z [M + H]+	Structural formula	Retention time RT, min
AV-6	$C_{19}H_{30}N_2O_4$	350.22056	351.22783		7.68
M1	$C_{18}H_{28}N_2O_4$	336.20491	337.21218		7.11
M2	$C_{18}H_{28}N_2O_3$	320.20999	321.21727		6.53

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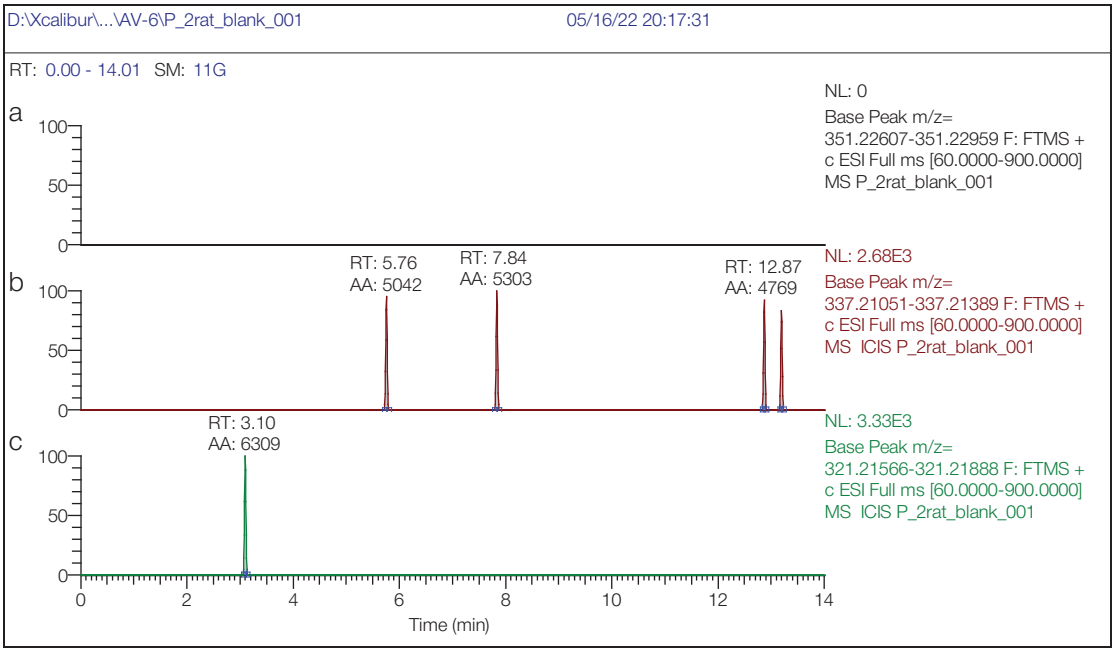

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Fig. 2. Rat plasma blank chromatogram: a — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 351,22783 (AV6); b — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 337,21218 (M1); c — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 321,21727 (M2)

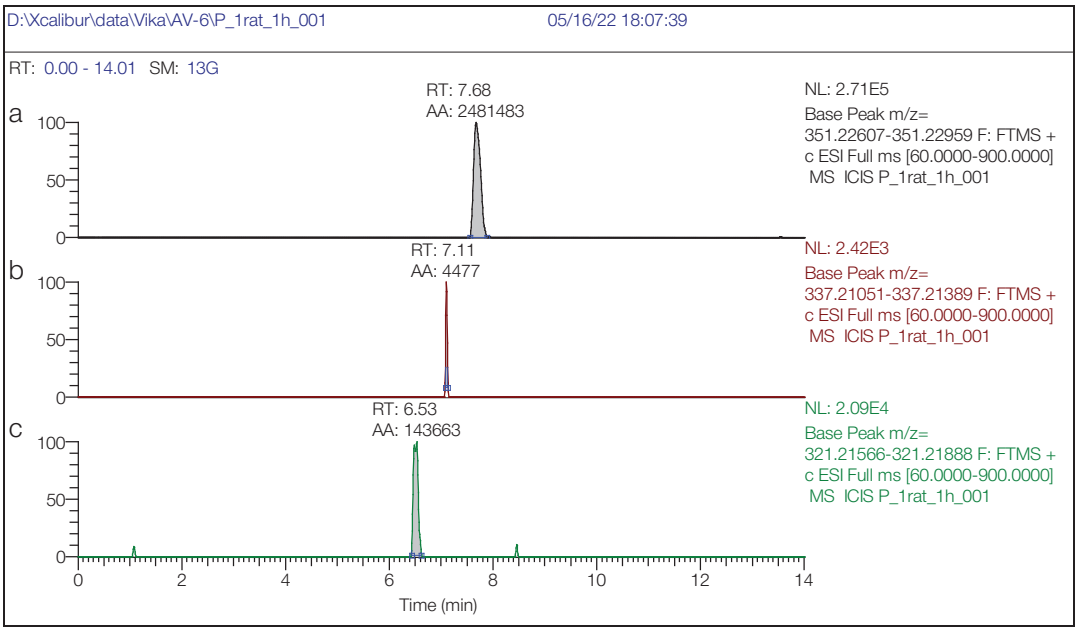

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Fig. 3. Rat plasma chromatogram 1 hour post single intragastric administration of AV6 (25 mg/kg bw): a — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 351,22783 (AV6); b — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 337,21218 (M1); c — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 321,21727 (M2)

The analysis of rat plasma chromatograms revealed the appearance of novel compounds and corresponding chromatographic peaks in samples collected 1 h post-administration (Fig. 3), which were identified as metabolites of AV6. Their structural characteristics are presented in Table 6.

The plasma chromatograms clearly demonstrate the presence of intact AV6 (Fig. 3a), along with trace

amounts of metabolite M1 (Fig. 3b) and metabolite M2 (Fig. 3c). The retention times (RT) were determined to be 7.68 min for AV6 and 6.53 min for metabolite M2.

The chromatographic comparison of rat plasma samples collected 1 h (Fig. 3) and 10 h (Fig. 4) after intragastric AV6 administration demonstrates significant kinetic changes. At the 1-h timepoint, the peak intensity of AV6 exceeded that of metabolite M2 by 20-fold. By 10 h post-administration, the intensity of the AV6 peak had decreased substantially, while the M2 metabolite peak showed marked intensification.

The obtained chromatographic data confirm the consistent detection of metabolite M2 throughout the 1–10 h period following AV6 administration. Notably, the relative abundance of M2 surpassed that of the parent compound by the 10-h observation point. This kinetic profile suggests a greater metabolic stability of M2 compared to AV6. Furthermore, the pharmacological effects originally attributed to AV6 may potentially be mediated by M2 activity. Metabolite M1 was not detected in plasma samples at either timepoint.

Results of AV6 and its metabolites quantification in urine samples

The mass chromatograms of urine samples collected before AV6 administration (Fig. 5), 8 h post-administration (Fig. 6), and 24 h post-administration (Fig. 7) demonstrate the following metabolic profile:

- in the blank sample (Fig. 5), no peaks corresponding to AV6 or its metabolites were detected, confirming the absence of background interference;
- by 8 h post-administration (Fig. 6), chromatograms revealed detectable peaks for both AV6 and its two metabolites (M1 and M2);
- the peak intensity (area) of AV6 was only slightly greater than those of the metabolites, indicating significant excretion of the parent compound in urine;
- at the 24-h timepoint (Fig. 7), AV6 was present only in trace amounts, while metabolites M1 (Fig. 7b) and M2 (Fig. 7c) remained clearly detectable.

No target compounds (AV6 or its metabolites M1/M2) were detected in blank (pre-dose) samples.

CONCLUSION

The conducted preclinical study to evaluate the acute toxicity, metabolism, and pharmacokinetics of AV6 represents a crucial step in translating previous findings on the antitumor potential of this derivative of natural phaeosphaeride A and advancing *in vivo* research. The investigated derivative of natural phaeosphaeride A — AV6 — can be classified as hazard class 3. No statistically significant changes were observed in the internal organs of experimental animals compared to the control group.

As a result of this study, a quantitative determination procedure for AV6 based on HPLC–MS/MS analysis has been developed. For the first time, data on metabolites formed in rats have been obtained. The pharmacokinetic

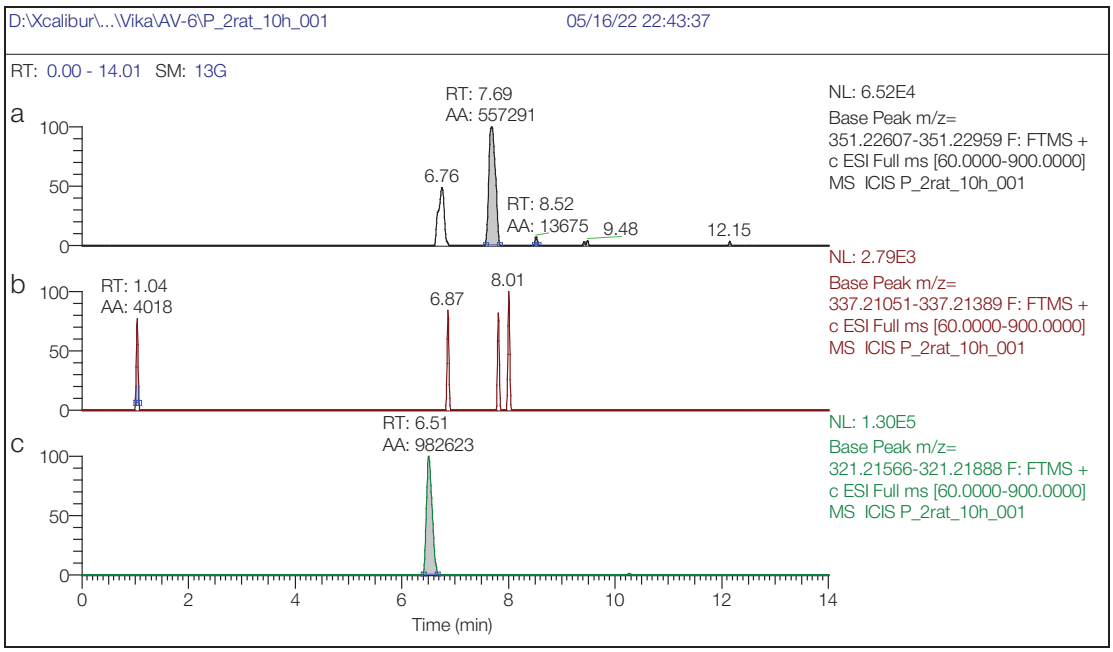

Figure prepared by the authors using their own data

Fig. 4. Chromatogram of rat plasma 10 h after single intragastric administration of AV6 (25 mg/kg bw): a — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 351,22783 (AV6); b — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 337,21218 (M1); c — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 321,21727 (M2)

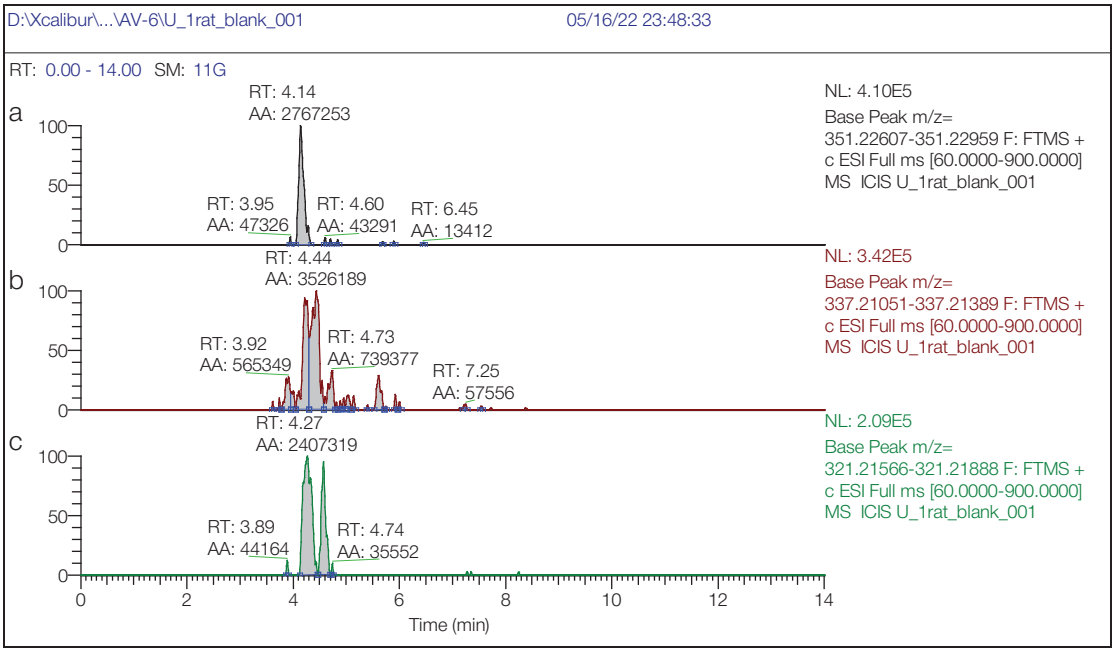

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Fig. 5. Chromatogram of rat urine blank sample: a — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 351,22783 (AV-6); b — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 337,21218 (M1); c — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 321,21727 (M2)

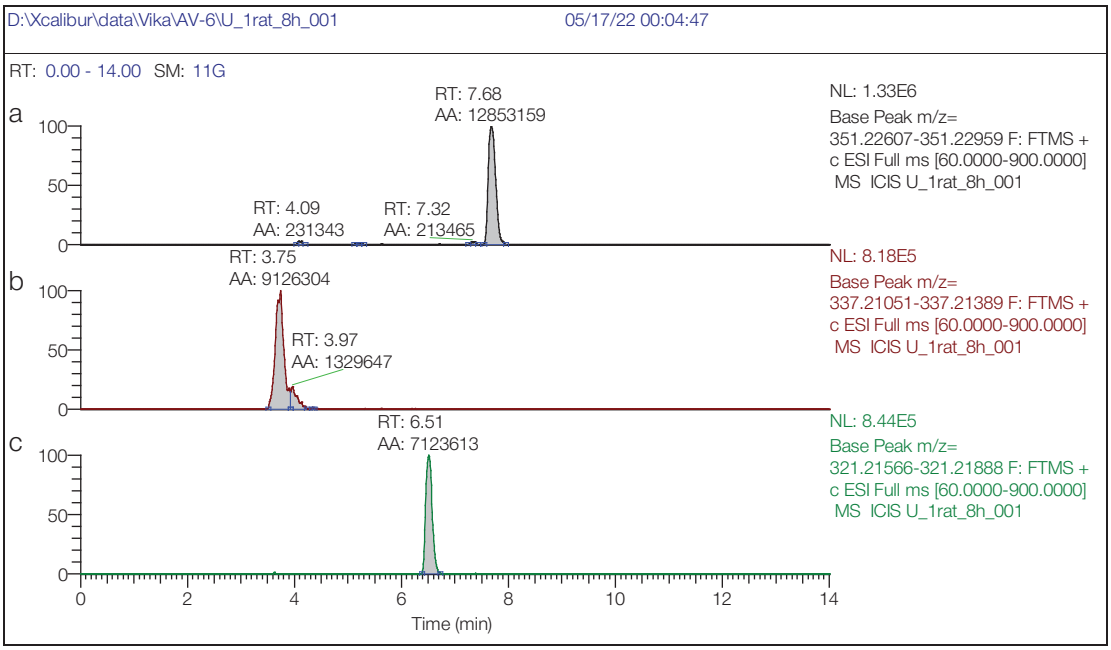

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Fig. 6. Chromatogram of a rat urine sample 8 h after a single intragastric administration of AV6 at a dose of 25 mg/kg bw: a — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 351,22783 (AV6), RT = 7.68 min; b — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 337,21218 (M1) RT = 3.75 min; c — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 321,21727 (M2) RT = 6.51 min

data of AV6 following intragastric administration in an oil-alcohol emulsion suggest that its pharmacokinetics can be formally described using a non-compartmental model. However, additional analysis of its distribution into hydrophobic compartments may be required, considering its physicochemical properties.

The data on AV6 metabolites obtained during the pharmacokinetic study in rats indicate that the metabolic

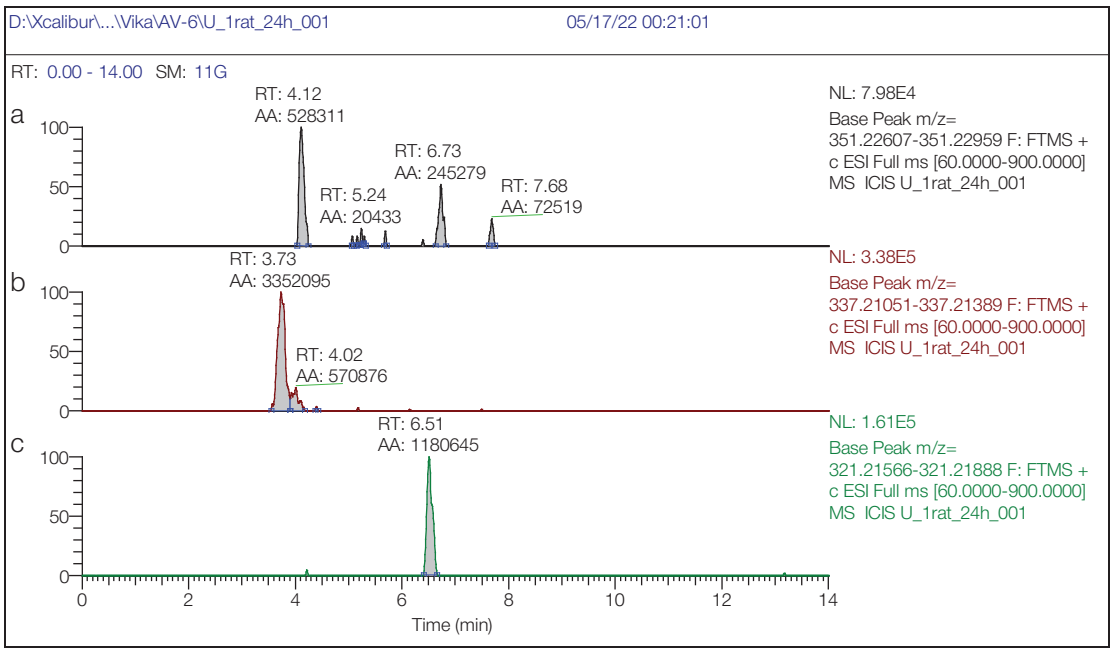

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Fig. 7. Chromatogram of rat urine sample 24 h post single intragastric administration of AV6 (25 mg/kg bw): a — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 351,22783 (AV6); b — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 337,21218 (M1); c — mass chromatogram reconstructed for exact ion mass [M + H] + c m/z 321,21727 (M2)

rate of the compound is relatively low and is primarily driven by chemical transformations at the nitrogen atom of the lactam ring. This results in the formation of metabolites that can be excreted in urine. The most likely mechanisms of these transformations include oxidative deacylation followed by hydrolysis.

References

1. Moten A, Schafer D, Farmer P, Kim J, Ferrari M. Redefining global health priorities: Improving cancer care in developing settings. *Journal of Global Health.* 2014;4(1):010304. https://doi.org/10.7189/jogh.04.010304
2. Delahaye C, Figarol S, Pradines A, Favre G, Mazieres J, Calvayrac O. Early steps of resistance to target therapies in non-small-cell lung cancer. *Cancer.*2022;14(11):2613. https://doi.org/10.3390/cancers14112613
3. Whitmire M, Ammerman J, de Lisio P, Killmer J, Kyle D, Mainstone E, et al. LC-MS/MS Bioanalysis Method Development, Validation, and Sample Analysis: Points to Consider When Conducting Nonclinical and Clinical Studies in Accordance with Current Regulatory Guidances. *Journal of Analytical and Bioanalytical Techniques.* 2011;1(1):S4–001. https://doi.org/10.4172/2155-9872.S4-001
4. Maloney KN, Hao W, Xu J, Gibbons J, Hucul J, Roll D, et al. Phaeosphaeride A, an inhibitor of STAT3-dependent signaling isolated from an endophytic fungus. *Organic Letters* 2006;8(18):4067–70. https://doi.org/10.1021/ol061556f
5. Abzianidze VV, Efimova KP, Poluektova EK, Trishin YuG, Kuznetsov VA. Synthesis and characterization of natural phaeosphaeride A and semi-natural phaeosphaeride B derivatives. *Mendeleev Communications.* 2017;27(5):490–2. https://doi.org/10.1016/j.mencom.2017.09.020
6. Abzianidze VV, Zakharenkova S, Kuznetsov VA, Beltyukov P, Moiseeva N, Mejia J, et al. Synthesis and Biological Evaluation of Phaeosphaeride A Derivatives as Antitumor Agents. *Molecules.* 2018;23(11):3043. https://doi.org/10.3390/molecules23113043
7. Abzianidze VV, Moiseeva NI, Suponina DS, Zakharenkova SA, Rogovskaya NYu, Laletina LA, et al. Natural Phaeosphaeride A Derivatives Overcome Drug Resistance of Tumor Cells and Modulate Signaling Pathways. *Pharmaceuticals.* 2022;15(4):395. https://doi.org/10.3390/ph15040395
8. Abzianidze VV, Kadochnikov VV, Suponina DS, Skvortsov NV, Beltyukov PP, et al. X-ray structure and in silico molecular docking of a natural phaeosphaeride A derivative for targets associated with kinase cascades. *Mendeleev Communications.* 2023;33:534–6. https://doi.org/10.1016/j.mencom.2023.06.030
9. Abzianidze VV, Beltyukov PP, Zakharenkova SA, Kuznetsov VA, Lukina DS, Ukolov AI, et al. *New phaeosphaeride derivatives with antitumor activity, method for obtaining and application of these compounds.* Patent of Russian Federation No. 2748533;2021 (In Russ.). EDN: HBRZRK
10. Abzianidze VV, Moiseeva NI, Beltyukov PP, Zakharenkova SA, Kuznetsov VA, Suponina DS, et al. *New phaeosphaeride derivatives with cytotoxic, antitumor activity and the ability to overcome drug resistance*. Patent of Russian Federation No. 2809986;2023 (In Russ.). EDN: LVBHXG
11. Bijsterbosch MK, Duursma AM, Bouma JM, Gruber M. The plasma volume of the Wistar rat in relation to the body

weight. *Experientia.* 1981;37(4):381–2. https://doi.org/10.1007/BF01959874
12. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology.* 2001;21(1):15–23. https://doi.org/10.1002/jat.727

Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria. The greatest contributions were made by Victoria V. Abzianidze, who supervised the study and approved the final version of the manuscript; Nikita V. Skvortsov, who worked with animals, assessed acute toxicity, performed initial analysis, and contributed to the acute toxicity section of the manuscript; Georgii V. Karakashev, who conducted the pharmacokinetic study of AV6 and identified its metabolites, contributed to the pharmacokinetic section of the manuscript, and edited it; Petr P. Beltyukov — animal handling and manuscript editing; Diana S. Suponina — AV6 substance handling; Valeriya O. Musatova — logistics; Alexander S. Bogachenkov — conceptualization and data analysis; Denis V. Krivorotov — manuscript editing.

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ANTICONVULSANT ACTIVITY OF ORIGINAL VALPROIC ACID AMINOETHERS IN CHOLINESTERASE INHIBITOR POISONING

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Introduction. Cholinesterase inhibitors present in household chemicals, agrochemicals, and a number of medicinal products represent the most common cause of acute intoxications accompanied by the development of convulsive syndrome. Delayed and repeated administration of existing antidotes proves ineffective. Compounds that are promising for the development of alternative therapeutic agents include derivatives of valproic acid.

Objective. Evaluation of the anticonvulsant efficacy of original valproic acid aminoethers in intoxication with phenylcarbamate as a cholinesterase inhibitor.

Materials and methods. Experiments were conducted using outbred white male rats aged 3 months with a body weight of 200–240 g. The tabular express method by Prozorovsky was used to determine the median lethal doses of the new compounds. To model the convulsive syndrome, phenylcarbamate was administered intraperitoneally to male rats at a dose of 1 mg/kg bw. The anticonvulsant activity of valproic acid aminoethers — N-methyl-4-piperidinol (VAA), quinuclidinol (QVA), and tropinol (TVA) — was assessed. The preparations were administered at doses of 21.5 mg/kg bw and 43 mg/kg bw after the onset of convulsions. The study was conducted using four experimental groups: phenylcarbamate — P (n = 8), P+VAA (n = 16), P+TVA (n = 16), and P+QVA (n = 16). The test substances were dissolved in 0.9% sodium chloride solution and administered intraperitoneally, taking interspecies dose conversion into account. The volume of the intraperitoneally administered solution was 0.1 mL/100 g. The severity of the convulsive syndrome in the experiment was assessed using the Racine scale. The following efficacy indicators were taken into account: latent period, severity and duration of convulsive syndrome, and mortality. Statistical processing of the research results was performed using the Statistica 13.0 software package (Statsoft, USA).

Results. The established LD_{50} values of the original valproic acid aminoethers under study correspond to class 3 of moderately toxic substances. At a dose of 21.5 mg/kg bw, the proportion of rats with severe convulsions significantly decreased in all groups; the fastest anticonvulsant effect was recorded in the QVA group (after 10 min, convulsions were absent). The efficacy of VAA and TVA at a dose of 43 mg/kg bw was comparable to the dose of 21.5 mg/kg bw; in the QVA group, the proportion of animals with convulsions remained high after 10 min. A significant reduction in the duration of convulsions was revealed in the QVA group at doses of 21.5 mg/kg bw and 43 mg/kg bw. A significant decrease in the intensity of convulsions was detected in the VAA and QVA groups at a dose of 21.5 mg/kg bw, and at a dose of 43 mg/kg bw in the VAA and TVA groups.

Conclusions. The new aminoethers of valproic acid exhibit anticonvulsant activity in intoxication with a reversible cholinesterase inhibitor. At a dose of 21.5 mg/kg bw, QVA is the most effective; however, at a dose of 43 mg/kg bw, manifestations of toxicity are observed and VAA is more effective. Despite animal mortality, TVA also demonstrates its efficacy at a dose of 43 mg/kg bw.

Keywords: valproic acid aminoethers; convulsive syndrome; cholinesterase inhibitors; carbamates; anticonvulsant therapy

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ПРОТИВОСУДОРОЖНАЯ АКТИВНОСТЬ ОРИГИНАЛЬНЫХ АМИНОЭФИРОВ ВАЛЬПРОЕВОЙ КИСЛОТЫ ПРИ ИНТОКСИКАЦИИ ИНГИБИТОРОМ ХОЛИНЭСТЕРАЗ

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Введение. Наиболее распространенной причиной острых интоксикаций, сопровождающихся развитием судорожного синдрома, являются ингибиторы холинэстераз в составе бытовой химии, агрохимикатов и ряда лекарственных средств. Отсроченное

и повторное применение существующих антидотов малоэффективно. К соединениям, перспективным для разработки альтернативных средств терапии, относятся производные вальпроевой кислоты.

Цель. Оценка противосудорожной эффективности оригинальных аминоэфиров вальпроевой кислоты при интоксикации ингибитором холинэстеразы — фенилкарбаматом.

Материалы и методы. Эксперименты проведены на беспородных белых крысах-самцах возрастом 3 месяца и массой тела 200–240 г. При определении средних летальных доз новых соединений использовали табличный экспресс-метод по В.Б. Прозоровскому. Для моделирования судорожного синдрома внутрибрюшинно вводили крысам-самцам фенилкарбамат в дозе 1 мг/кг м.т. Оценивали противосудорожную активность аминоэфиров вальпроевой кислоты: N-метил-4-пиперидинольный (АВК), хинуклидинольный (ХАВК) и тропиновый (ТАВК), вводимые в дозах 21,5 и 43 мг/кг м.т. после начала судорог. Исследование проведено на 4 опытных группах: фенилкарбамат «Ф» (n = 8), Ф+АВК (n = 16), Ф+ТАВК (n = 16), Ф+ХАВК (n = 16). Исследуемые субстанции растворяли в 0,9%-ном растворе хлорида натрия и вводили внутрибрюшинно, с учетом межвидового пересчета доз. Объем вводимого внутрибрюшинно раствора составлял 0,1 мл/100 г. Выраженность судорожного синдрома в эксперименте оценивали по шкале Racine. Учитывали показатели эффективности: латентный период, выраженность и продолжительность судорожного синдрома, летальность. Статистическую обработку результатов исследования производили с помощью пакета программы Statistica 13.0 (Statsoft, США).

Результаты. Установленные значения $ЛД_{50}$ оригинальных аминоэфиров вальпроевой кислоты соответствуют 3-му классу умеренно токсичных веществ. В дозе 21,5 мг/кг м.т. значимо уменьшалась доля крыс с выраженными судорогами во всех группах, наиболее быстрый противосудорожный эффект регистрировали в группе ХАВК (через 10 мин судороги отсутствовали). Эффективность АВК и ТАВК при использовании в дозе 43 мг/кг м.т. была сопоставима с дозой 21,5 мг/кг м.т., в группе ХАВК через 10 мин доля животных с судорогами оставалась высокой. Достоверное уменьшение продолжительности судорог выявлено в группе ХАВК в дозах 21,5 и 43 мг/кг м.т. Достоверное снижение интенсивности судорог выявлено в группах АВК и ХАВК в дозе 21,5 мг/кг м.т., группах АВК и ТАВК — в дозе 43 мг/кг м.т.

Выводы. Новые аминоэфиры вальпроевой кислоты проявляют противосудорожную активность при интоксикации обратимым ингибитором холинэстераз. В дозе 21,5 мг/кг м.т. наиболее эффективен ХАВК, однако в дозе 43 мг/кг м.т. наблюдаются проявления токсичности и более эффективен АВК. Несмотря на летальность животных, ТАВК также проявляет свою эффективность в дозе 43 мг/кг м.т.

Ключевые слова: аминоэфиры вальпроевой кислоты; судорожный синдром; ингибиторы холинэстераз; карбаматы; противосудорожная терапия

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INTRODUCTION

Poisoning with cholinesterase inhibitors remains the primary cause of generalized convulsive syndrome of toxic etiology. Substances in this group include irreversible inhibitors, primarily organophosphorus compounds (OPCs), and reversible inhibitors, such as derivatives of carbamic acid (carbamates). Carbamates and OPCs are used as components of agricultural and household chemicals (pesticides, insecticides), as well as plasticizers and polymeric materials. Carbamates are also widely applied in the pharmaceutical industry as part of medicinal products [1, 2]. The number of victims of poisoning with cholinesterase inhibitors (household poisonings, agrochemical poisonings, drug overdoses, or suicides) amounts to several million per annum worldwide.

The toxic effects of cholinesterase inhibitors on the body are manifested in the development of miosis, bronchospasm, hypersecretion, vomiting, arrhythmia, and respiratory failure. These conditions result from enhanced muscarinic and nicotinic stimulation due to the inhibition of acetylcholinesterase (AChE) activity. This inhibition promotes the accumulation of acetylcholine in neuronal synapses and the development of cholinergic syndrome [3]. Additionally, butyrylcholinesterase, carboxylesterase, and some other enzymes are inhibited [1, 4, 5]. At the level of the central nervous system (CNS), intoxication effects include generalized convulsive seizures, which can last for more than 30 min and involve significant damage to neurons and neuroglial cells in the brain. Furthermore, cholinergic manifestations are supplemented by glutamatergic excitotoxicity, progression of neuroinflammation and neurodegeneration. Victims

may experience persistent neurological disorders for an extended period of time [6].

It appears evident that a timely and effective suppression of convulsions is essential for improving the survival rates after acute poisoning with cholinesterase inhibitors, as well as for ensuring neuroprotection and prevention of CNS dysfunction in the long-term post-intoxication period. However, the existing antidote therapy is primarily aimed at reducing severity and preventing fatalities in the earliest stages of intoxication (prior to the onset of convulsions).

The effectiveness of antidote therapy decreases significantly when administered during actively developing or established convulsive syndrome. In particular, atropin [5] acts only on muscarinic, rather than on nicotinic, cholinergic receptors [6]. This compound is most effective when administered prophylactically or within the first minutes after exposure. Oximes, such as pralidoxime, etc., are recommended as prophylactic antidotes and exclusively for organophosphorus compound (OPC) poisoning [4]. Among benzodiazepines, which interact with gamma-aminobutyric acid (GABA) receptors, midazolam is preferred due to its rapid action, attributed to its high penetrative properties across the blood–brain barrier (BBB) [5]. However, their efficacy may be significantly reduced due to a substantial decline in the expression of receptor subunits and structurally associated proteins and enzymes capable of interacting with these substances within 10–20 min after exposure [6]. This greatly increases the share of refractory (antidote-resistant) convulsions.

The above-mentioned considerations underscore the necessity for developing new effective means to suppress convulsive syndrome in poisoning with cholinesterase inhibitors. One promising direction consists in the development of anticonvulsant agents based on derivatives of valproic acid. It is known that valproic acid participates in pre- and postsynaptic modulation of GABAergic signaling, affects sodium, calcium, and potassium channels, can increase extracellular levels of serotonin and dopamine in the hippocampus, modulate neurogenesis, and exert a neuroprotective effect [7]. However, the high effective dose of this anticonvulsant for generalized convulsive syndrome (over 150 mg/kg bw) limits its use as an antidote. Moreover, high doses of valproic acid are associated with a teratogenic effect [8].

With the purpose of reducing the effective dose, new pharmaceutical agents based on valproic acid are being developed. For example, valpromide (VPM), a recently developed substance, was shown to act as a prodrug (valproic acid is released during hydrolysis in the stomach) [8, 9]. However, VPM, approved only in France and Italy (Depamide®), is recommended for use exclusively in bipolar disorders (not as an anticonvulsant) [9]. Valnoctamide (VCD) — an isomer of valpromide — with its own therapeutic activity (biotransformation with the release of valproic acid in the body is minimal) has been developed. Currently, VCD has successfully passed phase IIb of clinical trials and is included in the list of sedative agents as an anticonvulsant [8]. Sec-butylpropylacetamide (SPD), a recently synthesized homologue of VCD, showed high anticonvulsant activity in various experimental models in preclinical studies, including cases of benzodiazepine-resistant convulsions [8]. The efficacy of SPD is due to a significantly faster rate of penetration through the blood–brain barrier (12 times faster than valproic acid). SPD was demonstrated to preserve cognitive functions and reduce neuronal damage [10]. However, SPD and VCD are poorly soluble in water and are used in the form of an emulsion [11], which is inconvenient for use as an antidote. All the above-mentioned derivatives of valproic acid are not registered as agents for the relief of generalized convulsive syndrome.

The aim of the study is a comparative investigation of the anticonvulsant efficacy of original valproic acid aminoether substances in poisoning with a cholinesterase inhibitor — phenylcarbamate.

MATERIALS AND METHODS

Original valproic acid derivatives were developed and synthesized at the Laboratory of Drug Synthesis of the Golikov Federal Research Center of Toxicology. The list and structural formulas of the tested substances are presented in Table 1.

In the experiments, outbred white male rats aged three months and weighing 200–240 g were used as the test system. The animals were obtained from the nursery of the National Research Center “Kurchatov

Table 1. Molecular characteristics of valproic acid aminoethers

Name	Gross formula	Purity (%)
(1-Methylpiperidin-4-yl) 2-propylpentanoate hydrochloride (VAA)	$C_{14}H_{27}NO_2$*HCl	98.27
1-Azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate (QVA)	$C_{15}H_{27}NO_2$*HCl	98.03
8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate (TVA)	$C_{16}H_{29}NO_2$*HCl	98.09

Table compiled by the authors

Institute" — RAPPOLOVO Laboratory Animal Breeding Facility (Leningrad Oblast). The animals were kept under standard conditions in accordance with the rules[1].

At the first stage, an assessment of acute toxicity and determination of the median lethal dose (LD_{50}) for TVA and QVA was conducted using the express method by Prozorovsky [12]. In order to study each compound, the experimental animals were divided into four groups of two rats each; a single intraperitoneal injection of the test solutions was performed (the substances were dissolved in 0.9% sodium chloride solution). The following doses were selected for the acute toxicity study of TVA and QVA substances: QVA — 63.1, 79.4, 100.0, 126.0 mg/kg; TVA — 79.4, 100.0, 126.0, 158.0 mg/kg. Previous studies had determined the LD_{50} for VAA to be 170 ± 1.2 mg/kg bw [13].

At the second stage, the pharmacological activity of the substances was studied using a model of convulsions induced by a reversible cholinesterase inhibitor — substituted 2[(dimethylamino)methyl] aryldimethylcarbamate hydrochloride (hereinafter referred to as phenylcarbamate) [14]. Phenylcarbamate was administered as a single intraperitoneal injection at a dose of 1 mg/kg bw [15]. The following experimental groups were formed: a group with isolated administration of phenylcarbamate "P" as the convulsive agent (n = 8), and three groups with administration of "P" followed by administration of the test corrective agents: P+VAA (n = 16), P+TVA (n = 16), P+QVA (n = 16). The anticonvulsant efficacy of the VAA, QVA, and TVA substances was assessed at two doses — 21.5 mg/kg bw and 43.0 mg/kg bw (eight animals per dose for each test substance).

The selection of effective doses of the test substances and the administration regimen was based on the dosage and usage protocols of sodium valproate (Convulex®) adopted in clinical practice for humans. The average dose of 7 mg/kg bw was used as the basis for calculating the effective dose. Interspecies dose conversion from human to rat was performed using the standard recommendations of Mironov[2]. The test dose values were calculated using the following coefficients: the therapeutic dose for rats was 7.0 × 39 (coefficient for a human weighing 70 kg) / 6.5 (coefficient for a rat weighing 200 g) ≈ 43.0 mg/kg bw.

The test substances were dissolved in a 0.9% sodium chloride solution and administered intraperitoneally, taking interspecies dose conversion into account. The volume of the intraperitoneally administered solution was 0.1 mL/100 g. Administration was performed within the first minutes after the onset of seizures at levels 3–4 on the Racine scale [16], which were induced by the administration of the cholinesterase inhibitor.

The following efficacy indicators were taken into account: latent period, severity and duration of convulsive syndrome, and mortality. Observation and recording of lethal outcomes were conducted over a 24-h period. Mortality was assessed based on the proportion of deceased rats relative to their total number in the study group after the administration of the convulsive agent during the 24-h observation period. The severity of convulsive syndrome in the experiment was assessed using the Racine scale. Seizures of level 4 and above, equivalent to generalized clonic-tonic seizures in humans, were classified as severe. The duration of convulsive syndrome was measured in minutes.

To assess the significance of differences in the frequency of rats exhibiting severe seizures, Fisher's exact test was used. A comparative assessment of the convulsive syndrome indicators in male rats poisoned with phenylcarbamate was performed using the Kruskal–Wallis test. To identify differences between individual groups, as well as between the studied substances and the "P" group, Dunn's multiple comparison test (post-hoc analysis) was employed. Statistical processing of the research results was carried out using the Statistica 13.0 software package (Statsoft, USA).

RESULTS

In the course of work to determine the quantitative characteristics of acute toxicity upon intraperitoneal administration of QVA, the following distribution of rat mortality in each dose subgroup was established: 0, 0, 2, 2 individuals, which allowed for the determination of the LD_{50} for QVA at a level of 89.8 ± 7.1 mg/kg; upon intraperitoneal administration of TVA — 0, 0, 2, 2 individuals, which corresponded to an LD_{50} of 113.1 ± 8.9 mg/kg. Based on the obtained LD_{50} values of the original valproic acid aminoethers, the compounds can be classified as class 3 moderately toxic substances.

The experimental model of seizures induced by a reversible acetylcholinesterase inhibitor (phenylcarbamate) [12, 13] following the administration of VAA, QVA, and TVA at two doses (21.5 mg/kg bw and 43.0 mg/kg bw) revealed no statistically significant difference between the share of deceased rats in the group of animals receiving the convulsive agent, regardless of the dose and the recorded time interval. During the experiment, the death of one animal in the "P" group at the 30-min observation mark, the death of one rat following the administration of TVA at a dose of 21.5 mg/kg bw at the 10-min observation mark, and the death of three rats in the QVA group at a dose of 43.0 mg/kg bw at the 20-min observation mark were recorded.

After the administration of phenylcarbamate, the number of rats with severe convulsions in the "P" group began to decrease from the 30-min observation point by 14%; by 70 min, convulsions were no longer recorded in the animals.

[1] SP 2.2.1.3218-14 dated September 28, 2014 "Sanitary and Epidemiological Requirements for the Design, Equipment, and Maintenance of Experimental-Biological Clinics (Vivaria)".

[2] Mironov AN. Guidelines for conducting preclinical drug trials. Part 1. Moscow: Grif&K; 2012.

Table 2. Effect of the investigated drugs on the occurrence of seizures in rats following administration of phenylcarbamate and valproic acid aminoether substances

Group	Time intervals, min								
	0	5	10	20	30	40	50	60	70
Number of rats (*n/N*) after administration of substances at a dose of 21.5 mg/kg b.w.									
P	8/8	8/8	8/8	8/8	6/7	5/7	3/7	1/7	0/7
P+VAA	8/8	8/8	3/8*	0/8*	0/8*	0/8*	0/8	0/8	0/8
P+TVA	8/8	8/8	0/7*	3/7*	1/7*	0/7*	0/7	0/7	0/7
P+QVA	8/8	8/8	0/8*	0/8*	0/8*	0/8*	0/8	0/8	0/8
Number of rats (*n/N*) after administration of substances at a dose of 43.0 mg/kg b.w.									
P	8/8	8/8	8/8	8/8	6/7	5/7	3/7	1/7	0/7
P+VAA	8/8	8/8	2/8*	0/8*	0/8*	0/8*	0/8	0/8	0/8
P+TVA	8/8	8/8	1/8*	1/8*	0/8*	0/8*	0/7	0/7	0/7
P+QVA	8/8	8/8	8/8	0/8*	0/8*	0/8*	0/8	0/8	0/8

Table compiled by the authors based on their own data

Note: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate; *n* — number of rats with severe convulsions; *N* — total number of rats in the group; * — differences are statistically significant compared to the P group ($p \leq 0.05$).

The administration of the original aminoethers reduced the number of animals with convulsion severity of level 4 and above (Racine scale), which corresponds to a generalized convulsive syndrome in humans (Table 2).

Following the administration of the test substances at a dose of 21.5 mg/kg bw, a statistically significant reduction in the proportion of rats with severe convulsions was observed during the 10–40 min period of observation (Table 2).

In the P+QVA group, animals with severe convulsions were completely absent starting from the 10-min observation mark, whereas in the other two groups, the anticonvulsant effect occurred later. Specifically, in the P+TVA group, no severe convulsions were observed at 10 min; however, cases of clonic-tonic convulsions were recorded over the next 20 min, which ceased by 40 min. In the P+VAA group, a reduction in convulsion severity occurred by the 20-min observation mark.

No statistically significant intergroup differences were identified when comparing animals that received different substances at the same time interval.

When the substances were administered at a dose of 43.0 mg/kg bw, the anticonvulsant efficacy in the P+VAA and P+TVA groups generally coincided with that established at the lower dose. However, in the P+QVA group, 10 min after substance administration, the number of animals with severe convulsions did not decrease compared to the “P” group. At the same time, by 10 min, in the P+VAA and P+TVA groups, the number of animals with convulsive syndrome was statistically significantly lower than that in the group with isolated administration of phenylcarbamate.

When assessing the total duration of convulsions using statistical processing of the results with the Kruskal–Wallis test, the presence of statistically significant differences between all studied groups upon administration of the substances at both doses was revealed (Table 3).

When constructing a quadratic matrix of post-hoc comparisons (*p*-values) for the duration of convulsions following intraperitoneal administration of the test substances and in the phenylcarbamate group using Dunn’s post-hoc test, no statistically significant intergroup differences were identified. However, when comparing the duration of convulsions in the P+QVA group (dose 21.5 mg/kg bw) with the values in the “P” group, a statistically significant reduction in the median (Me) duration of convulsions by 77% was detected ($p = 0.001$). A lower median duration of convulsions by 57% was also recorded when comparing the indicator in the P+QVA and P+VAA groups at the dose level of 21.5 mg/kg bw ($p = 0.041$).

When the substances were administered at a dose of 43.0 mg/kg bw, differences in the duration of convulsions were established, comparable to the results obtained when studying the effects of lower doses. Specifically, significant differences were found when

Table 3. Comparative assessment of convulsion duration in rats after intraperitoneal administration of phenylcarbamate and valproic acid aminoether substances at doses of 21.5 and 43.0 mg/kg bw

Group	Dose of valproic acid aminoethers	Animals amount	Convulsion duration, min			*p*-value of intergroup differences[1]
		N	M_e	Min	Max	
P	–	8	87.0	13.0	103.0	0.003
P+VAA	21.5 mg/kg b.w.	8	46.5	34.0	57.0	
P+TVA		8	40.0	8.0	44.0	
P+QVA		8	20.0	20.0	20.0	
P	–	8	87.0	13.0	103.0	0.002
P+VAA	43.0 mg/kg b.w.	8	70.0	31.0	81.0	
P+TVA		8	48.0	45.0	50.0	
P+QVA		8	30.0	12.0	32.0	

Table compiled by the authors based on their own data

Note: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate; «–» — isolated administration of phenylcarbamate; [1] — *p*-value in the Kruskal-Wallis test.

Table 4. Comparative assessment of the time-weighted sum of seizure intensity scores in male rats after intraperitoneal administration of valproic acid aminoether substances at doses of 21.5 and 43.0 mg/kg bw

Group	Dose of valproic acid aminoethers	Number of animals	Time-weighted sums of seizure intensity scores, score/min			p-value of intergroup differences[1]
			M_e	min	max	
P	–	7	270	210	310	0.001
P+VAA	21.5 mg/kg bw	8	61	53	98	
P+TVA		7	85	48	115	
P+QVA		8	19	18	20	
P	–	7	270	210	310	0.001
P+VAA	43.0 mg/kg bw	8	110	63	183	
P+TVA		8	66	65	165	
P+QVA		5	78	78	90	

Table compiled by the authors based on their own data

Note: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate; «–» — isolated administration of phenylcarbamate; [1] – *p*-value in the Kruskal–Wallis test.

comparing the P+QVA group with the phenylcarbamate group ($p = 0.001$) and in the intergroup comparison between P+QVA and P+VAA ($p = 0.039$).

Time-weighted sums of seizure intensity scores (area under the curve "seizure score–time," AUC) were calculated. Deceased individuals were excluded from the calculations. It was demonstrated (Table 4) that there are statistically significant differences between the AUC indicators upon administration of the substances at doses of 21.5 mg/kg bw ($p = 0.001$) and 43.0 mg/kg bw ($p = 0.001$).

Upon administration of the substances at a dose of 21.5 mg/kg bw, the time-weighted sums of seizure intensity scores in the P+VAA group were found to be statistically significantly lower by 77% than the corresponding indicator in the phenylcarbamate group ($p = 0.041$), and in the P+QVA group — by 93% ($p = 0.001$) based on the median, respectively. In the P+TVA group, the

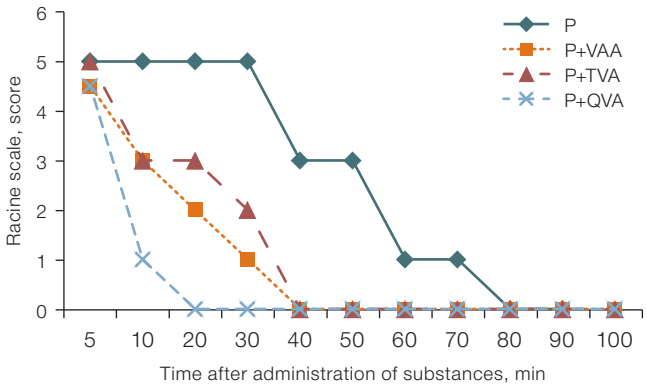

Figure prepared by the authors based on their own data

Fig. 1. Median-based plots of the severity of the convulsive syndrome according to the Racine scale (points) versus time after administration of valproic acid aminoether substances at a dose of 21.5 mg/kg bw to male rats: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate

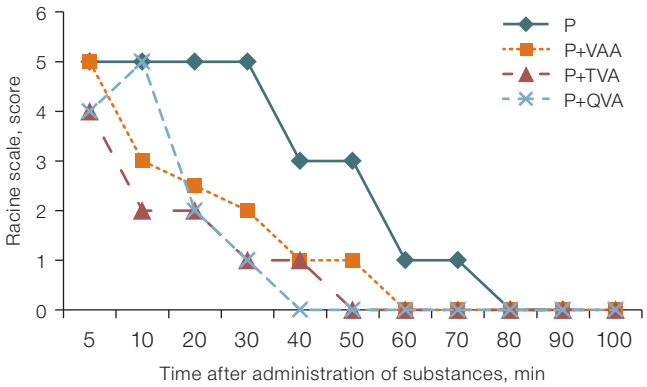

Figure prepared by the authors based on their own data

Fig. 2. Median-based plots of the severity of the convulsive syndrome according to the Racine scale (points) versus time after administration of valproic acid aminoether substances at a dose of 43 mg/kg bw to male rats: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate

AUC level was lower by 84%, although not reaching statistical significance compared to the "P" group. A statistically significant decrease in the AUC indicator was also recorded in the P+QVA group compared to the P+TVA group, with a difference of 78% (p = 0.026).

When the substances were administered at a dose of 43.0 mg/kg bw, a significant reduction in the median AUC indicator was noted: by 59% (p = 0.015) in the P+VAA group and by 79% in the P+TVA group (p = 0.001) compared to the animals that received only phenylcarbamate.

Figures 1 and 2 show the dynamics of the median scores for the severity of convulsive syndrome (from 0 to 6 points on the Racine scale) after administration of the test substances over a 100-min observation period. Administration of the QVA substance at a dose of 21.5 mg/kg bw demonstrated the most favorable time course for the severity of convulsive syndrome, corresponding to the greater anticonvulsant efficacy of this compound. Conversely, when a dose of 43.0 mg/kg bw was administered, more preferable changes were observed in animals that received VAA and TVA.

DISCUSSION

Our results indicate that the administration of original valproic acid aminoethers in the setting of acute intoxication with a cholinesterase inhibitor contributed to a pronounced reduction in the duration of convulsions within the first 10–20 min after the onset of exposure. The highest efficacy was demonstrated by QVA at a dose of 21.5 mg/kg bw. This dose led to cessation of convulsions in 100% of animals after 10 min.

The time-weighted sum of seizure intensity scores (AUC) in the groups receiving the original valproic acid aminoethers at both doses was significantly lower compared to the group of animals without therapy (only phenylcarbamate administration), indicating sufficient efficacy of the test substances in reducing the severity of not only intense but also other types of convulsions. Overall, the lowest median AUC value (corresponding to the highest efficacy) was established for QVA (valproic acid quinuclidinol aminoether) when administered at a dose of 21.5 mg/kg bw. The VAA and TVA samples at the studied doses were comparable to each other in terms of efficacy.

Although no statistically significant differences in mortality were detected between the studied groups across all time intervals, the identified death cases of three animals at the 20-min mark of the experiment following the use of QVA at a high dose (43.0 mg/kg bw) require further investigation. It is evident that these cases could have been associated with either the toxicity of QVA or the individual reaction of specific animals to phenylcarbamate exposure, independent of QVA administration. Furthermore, the pronounced efficacy of QVA at a dose of 21.5 mg/kg bw suggests that a further dose reduction without efficacy loss is possible and that the 43 mg/kg bw dose may be excessive. It has been previously established that the anticonvulsant efficacy of valpromide in experiments on mice is 3–5 times higher than that of valproic acid [9]; it is possible that in the case of QVA, the optimal dose will also be significantly lower and not associated with any manifestations of toxicity.

Previous animal models demonstrated that, in comparision with atropine — a standard antidote for intoxication with cholinesterase inhibitors [17], VAA is more effective in reducing the duration of convulsive syndrome. In this light, the developed original valproic acid derivatives should be considered promising for further study and the development of agents to terminate the convulsive syndrome induced by cholinesterase inhibitors, including irreversible ones.

CONCLUSIONS

1. The developed original valproic acid derivatives (VAA, QVA, and TVA) are effective in relieving convulsive syndrome developing due to poisoning with cholinesterase inhibitors and can be used for further study as potential medicinal products.

2. In terms of all the parameters studied, the QVA substance exhibited the highest anticonvulsant efficacy at a dose of 21.5 mg/kg bw. However, when using a dose of 43 mg/kg bw, the VAA substance demonstrated a more effective relief of the convulsive syndrome. The TVA substance exhibited its anticonvulsant efficacy when used at the increased dose of 43 mg/kg bw.

3. Further selection of effective doses and investigation of the toxicity of the valproic acid quinuclidinol aminoether (QVA) are necessary.

References

1. King AM, Aaron CK. Organophosphate and carbamate poisoning. *Emergency Medicine Clinics of North America*. 2015;33(1):133–51. https://doi.org/10.1016/j.emc.2014.09.010
2. Matošević A, Bosak A. Carbamate group as structural motif in drugs: a review of carbamate derivatives used as therapeutic agents. *Arhiv za Higijenu Rada I Toksikologiju*. 2020;71(4):285–99. https://doi.org/10.2478/aiht-2020-71-3466
3. Reddy BS, Skaria TG, Polepalli S, Vidyasagar S, Rao M, Vijayanarayana K, et al. Factors associated with outcomes in organophosphate and carbamate poisoning: a retrospective study. *Toxicological Research*. 2020;36(3):257–66. https://doi.org/10.1007/s43188-019-00029-x
4. Gladkih VD, Nazarov VB. Cholinesterase reactivators in the therapy of intoxication with neurotropic physiologically active substances. *Extreme Medicine*. 2014;1(47):54–6 (In Russ.). EDN: SAFQEV
5. Alozi M, Rawas-Qalaji M. Treating organophosphates poisoning: management challenges and potential solutions. *Critical Reviews in Toxicology*. 2020;50(9):764–79. https://doi.org/10.1080/10408444.2020.1837069
6. Reddy DS. Mechanism-based novel antidotes for organophosphate neurotoxicity. *Current Opinion in Toxicology*.2019;14:35–45. https://doi.org/10.1016/j.cotox.2019.08.001
7. Romoli M, Mazzocchetti P, D'Alonzo R, Siliquini S, Rinaldi VE, Verotti A, et al. Valproic Acid and Epilepsy: From Molecular Mechanisms to Clinical Evidences. *Current Neuropharmacology*. 2019;17(10):926–46 https://doi.org/10.2174/1570159X17666181227165722
8. Bialer M, Cross H, Hedrich UBS, Lagae L, Lerche H, Loddenkemper T. Novel treatment approaches and pediatric research networks in status epilepticus. *Epilepsy & Behavior*. 2019;101(Pt B):106564. https://doi.org/10.1016/j.yebeh.2019.106564
9. Delage C, Palayer M, Etain B, Hagenimana M, Blaise N, Smati J, et al. Valproate, divalproex, valpromide: Are the differences in indications justified? *Biomedicine & Pharmacotherapy*. 2023;158:114051. https://doi.org/10.1016/j.biopha.2022.114051
10. Mawasi H, Bibi D, Shekh-Ahmad T, Shaul C, Blotnik S, Bialer M. Pharmacokinetic-Pharmacodynamic Correlation and Brain Penetration of sec-Butylpropylacetamide, a New CNS Drug Possessing Unique Activity against Status Epilepticus. *Molecular Pharmaceutics*. 2016;13(7):2492–6. https://doi.org/10.1021/acs.molpharmaceut.6b00221
11. Haines KM, Matson LM, Dunn EN, Ardinger CE, Lee-Stubbs R, Bibi D, et al. Comparative efficacy of valnoctamide and sec-butylpropylacetamide (SPD) in terminating nerve agent-induced seizures in pediatric rats. *Epilepsia*. 2019;60(2):315–21 https://doi.org/10.1111/epi.14630
12. Prozorovsky VB. Statistical processing of pharmacological research results. *Psychopharmacology & Biological Narcology*. 2007;7(3–4):2090–120 (In Russ.). EDN: JVWCBJ
13. Prokopenko LI, Zaitseva MA, Bespalov AYa, Melekhova AS, Petrov AN, Belskaya AV, et al. *Hydrochloride (1-methylpiperidin-4-yl)-2-propylpentanoate, which has anticholinergic and anticonvulsant activity*. Patent of the Russian Federation No. 2714135; 2020 (In Russ.).
14. Bespalov AYa, Prokopenko LI, Gorchakova TL, Kozlov VK, Petrov AN, Zaitseva MA, et al. *Hydrochlorides of substituted 2-[(dimethylamino)methyl] aryldimethyl carbomates with anticholinesterase activity*. Patent of the Russian Federation. No. 2754133; 2021 (In Russ.).
15. Melekhova AS, Belskaya AV, Zorina VN, Melnikova MG, Kubarskaya LG, Gaikova ON. Experimental model of convulsive syndrome based on phenylcarbamate. *Extreme Medicine*. 2024;26(4):38–48 (In Russ.). https://doi.org/10.47183/mes.2024-26-4-38-48
16. Racine RJ Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalography and Clinical Neurophysiology*. 1972;32(3):281–94. https://doi.org/10.1016/0013-4694(72)90177-0
17. Melekhova AS, Petrov AN, Bespalov AY, Belskaya AV, Melnikova MV, Zatsepin EP, et al. Experimental pharmacotherapy of convulsive syndrome in the simulation of severe carbamate poisoning. *Medline.ru. Russian Biomedical Journal* 2019;20(2):294–306 (In Russ.). EDN: JRVAPQ

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HEALTH RISKS TO THE POPULATION ASSOCIATED WITH POISONING BY NEUROTROPIC TOXICANTS (ANALYTICAL REVIEW)

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Introduction. Issues related to the epidemiologic aspects and clinical manifestations of poisoning by neurotoxicants, whose effects cause serious harm to the health of victims, are highly relevant. Acute and chronic poisoning can be manifested through diverse patterns; however, regardless of the type of neurotoxicant, all victims experience asthenovegetative and psychoorganic syndromes. These syndromes can develop during both toxicogenic and somatogenic phases of poisoning, manifesting in the period of its long-term consequences.
Objective. Scientific substantiation of the risks of poisoning by neurotoxicants, which pose a serious threat to public health due to their systemic toxic effects and the development of multiorgan pathology, including at the stage of long-term consequences of poisoning.
Discussion. In Russia, among various types of acute poisoning, intoxications by substances causing primary damage to the central nervous system rank first. The routes of entry of neurotoxicants into the body are indicated, and the forms of manifestation of neurotoxic processes are described. The pathogenesis of the toxic action of organic solvents, heavy metal salts, barbiturates, and carbamates is analyzed. Toxic neurotropic substances can adversely affect the nervous system both directly and indirectly, through damage to other organs and systems. Clinical cases of acute poisoning by neurotoxicants are described. After poisoning by representatives of the group of neurotropic toxicants, after a certain period of time, the victim develops long-term consequences with a highly varying clinical picture.
Conclusions. The presented data on poisoning exposures to neurotoxicants demonstrate the clinical and pathogenetic significance of their effects not only on the central nervous system but also on other organs and tissues, the development of systemic pathological processes and multiorgan pathologies. The identified features of the toxic action must be taken into account when analyzing the health risks to victims of poisoning with neurotropic toxicants. The most significant manifestations of the effects on other organs/tissues should be reflected in the protocols for diagnosing the severity of such poisoning accidents and their long-term consequences, as well as in the use of metabolic and cytoprotective agents for their treatment.

Keywords: neurotoxicity; neurotoxicant; poisoning; methanol; barbiturates; heavy metals; lead; carbamates; 1,4-butanediol

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УДК 615.9

РИСКИ ДЛЯ ЗДОРОВЬЯ НАСЕЛЕНИЯ, СВЯЗАННЫЕ С ОТРАВЛЕНИЯМИ НЕЙРОТРОПНЫМИ ТОКСИКАНТАМИ (АНАЛИТИЧЕСКИЙ ОБЗОР)

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Введение. Вопросы эпидемиологии и особенностей клинической картины отравлений нейротропными токсикантами, действие которых наносит серьезный вред здоровью пострадавших, являются актуальными и приоритетными. Картина острых и хронических отравлений может быть весьма разнообразна, однако есть ведущие синдромы, такие как астеновегетативный и психоорганический, которые наблюдаются у пострадавших независимо от вида нейротоксиканта. Данные синдромы могут развиваться как в токсикогенную фазу отравления, так и в соматогенную, проявляясь в периоде отдаленных последствий отравлений.
Цель. Научное обоснование рисков отравлений нейротоксикантами, представляющими серьезную угрозу для здоровья населения, связанную с их системным токсическим действием и формированием полиорганной патологии, в том числе на этапе отдаленных последствий отравлений.
Обсуждение. Отмечено, что в картине острых отравлений в Российской Федерации первое место занимают интоксикации веществами, вызывающими первичное поражение ЦНС. Показаны пути поступления нейротоксикантов в организм, описаны формы проявления нейротоксических процессов и патогенез токсического действия органических растворителей, солей тяжелых металлов, барбитуратов и карбаматов. Установлено, что токсичные нейротропные вещества могут оказывать негативное воздействие

на нервную систему как напрямую, так и опосредованно, через повреждение других органов и систем. Представлены описания клинических случаев острых отравлений нейротоксикантами. Выявлено, что после отравления представителями группы нейротропных токсикантов через определенный период времени у пострадавшего развиваются отдаленные последствия и клиническая картина их может быть весьма разнообразна.

Выводы. Представленные сведения по отравлениям нейротоксикантами показывают клиническую и патогенетическую значимость их действия не только на ЦНС, но и на другие органы и ткани, развитие системных патологических процессов и полиорганной патологии. Выявленные особенности токсического действия необходимо учитывать при анализе рисков здоровью пострадавших от отравления нейротропными токсикантами, а наиболее значимые проявления действия на другие органы/ткани должны найти отражение в протоколах диагностики степени тяжести таких отравлений и их отдаленных последствий, а также в применении средств метаболического и цитопротекторного действия для их лечения.

Ключевые слова: нейротоксичность; нейротоксикант; отравление; метанол; барбитураты; тяжелые металлы; свинец; карбаматы; 1,4-бутандиол

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INTRODUCTION

The rapid development of chemical science has been accompanied by uncovering fundamental patterns of chemical processes, developing chemical compounds through targeted synthesis, and establishing large-scale chemical production, thereby integrating chemistry into virtually all spheres of human life, including industry, agriculture, military affairs, and everyday life. The scientific and technological progress has undoubtedly enhanced the standards of living; however, it has also brought about new risks leading to incidents and even technological disasters of various scales. Virtually all types of emergencies are related to the potential adverse effects of chemical agents on human life, health, and the environment. Unfortunately, the frequency of accidents at chemically hazardous facilities resulting in casualties continues to demonstrate a persistent trend [1].

In Russia, for a number of years, mortality from external causes has been ranking third after oncological and cardiovascular diseases. In the structure of mortality from external causes, every fourth case of death has been caused by acute poisoning of chemical etiology. Acute poisoning remains a serious medical and social problem, as evidenced by its regular occurrence and the resulting consequences, including high rates of fatalities and disability among the victims [2].

According to poisoning statistics in the Russian Federation for the 2022–2024 period, the number of food poisoning incidents decreased; however, the number of victims of all other monitored toxicants showed a growing trend (Table 1). In 2023, Solonin et al. conducted a retrospective observational study to evaluate the results of chemical and toxicological analysis in patients admitted to the Department of Acute Poisonings and Somatopsychiatric Disorders of the Sklifosovsky Institute for Emergency Medicine (SIEM) in 2019–2021. Thus, during 2019–2021, 9590 patients sought specialized toxicological care at the SIEM, which corresponded to approximately one case of poisoning with neurotropic toxicants per 1000 of the population [3].

In the structure of acute poisoning in the Russian Federation, intoxications by substances that primarily cause damage to the nervous system rank first. The share of poisoning cases by neurotropic toxicants was about 65%, with more than a third of cases classified as severe and extremely severe intoxications [4, 5].

Neurotoxicity is the ability of a toxic substance to affect the central nervous system (CNS), leading to the destruction of its structure and/or disruption of its function [6]. The toxic process can cause such disorders in the body as changes in energy metabolism, neuromuscular transmission problems, damage to cell membranes and synapses. Neurotoxicity can be direct, i.e., caused by the action of a toxic substance directly on the nervous system, or indirect, when toxicity arises due to damage to other organs and systems [7].

Neurotoxic processes can manifest as impairments in motor, sensory, and cognitive functions, as well as changes in the emotional state [8]. Depending on the conditions of exposure, the structure of the toxicant, and its neurotoxic potential, the developing processes can be acute or chronic [6].

Table 1. Distribution of poisoning cases of chemical etiology by main monitored groups in the Russian Federation for 2022–2024

Toxicants groups	Analyzed period (year)					
	2022		2023		2024	
	total (people)	of which fatal (people)	total (people)	of which fatal (people)	total (people)	of which fatal (people)
Alcohol-containing products	30,917	9228	32,540	10,013	30,321	9313
Narcotic substances	22,054	7077	25,188	7909	23,289	6546
Medicinal products	20,940	662	23,748	710	24,610	761
Food products	1549	27	1536	18	1174	15
Other monitored species	25579	5981	29671	6197	30259	6287
Total	101,039	22,975	112,683	24,847	109,653	22,922

Table prepared by the authors based on data from the internet source[1]

The list of substances that can cause a chronic neurotoxic process is quite extensive, with the most common being:

- toxicants present in the external environment (derivatives of mercury, lead, arsenic, etc.);
- pharmaceuticals (antidepressants, antipsychotics, antiepileptic drugs, cytostatics, antibiotics, synthetic antimicrobial agents, etc.);
- organic solvents (benzene, toluene, acetone, ethyl, methyl, propyl alcohols, etc.);
- industrial chemicals and toxic compounds (organophosphorus and organochlorine pesticides, carbamates, pyrethroids, defoliants and other agrochemical agents, carbon monoxide, etc.) [9].

As a result of poisoning exposure to neurotoxicants, persistent impairments in mental and physical activity, emotional state, cognitive processes, sensitivity, as well as focal neurological disorders may occur. The available literature shows that while the initial stages of pathogenesis and the clinical development of acute poisoning have been extensively studied, the long-term consequences of poisoning by neurotoxicants require elucidation. In addition, data on the statistics of delayed nervous system disorders are lacking. CNS lesions developing in the long-term period after acute intoxication are usually not associated with these poisoning incidents[2].

In this work, our aim is to scientifically substantiate the risks of poisoning by neurotoxicants, which pose a serious threat to public health due to their systemic toxic effects and the development of multiorgan pathologies, including at the stage of long-term consequences of poisoning.

MATERIALS AND METHODS

A literature search was conducted in electronic bibliographic databases, including the Russian Science Citation Index (RSCI), Scopus, Web of Science (WoS), and PubMed. Search queries included the following keywords: neurotoxicity, intoxications by neurotropic toxicants, severe poisonings, long-term consequences. The review covers publications with an information search depth of no more than five years, as well as literature sources that are considered fundamental works on the subject under discussion, regardless of their publication year.

RESULTS AND DISCUSSION

In order to fulfill the research objectives, we carry out a detailed examination of representative substances from each group that can trigger a chronic neurotoxic process.

[1] Information on acute poisonings of chemical etiology by constituent entities of the Russian Federation. Federal Center for Hygiene and Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing. https://fcgie.ru/sgm.html (access date: 10.04.2025).

[2] Methodological Recommendations (MR) FMBA of Russia 12.05-18. Clinical Presentation, Diagnosis, Prevention and Treatment of Central Nervous System Lesions Following Severe Poisonings with Neurotoxicants. Moscow; 2018.

Environmental pollutants (heavy metals)

The term "heavy metals" (HMs) characterizes a broad group of substances that enter the environment primarily as a result of human activity [10, 11]. Sources of HM pollution include industrial and agricultural production, chemical plants, waste incineration facilities, and boiler houses. Additionally, there exist non-anthropogenic sources of HM pollution, such as volcanic eruptions [12]. Accumulation of HMs occurs in polluted air, water, soil, and consumer goods (e.g., cosmetic products) [13].

There are two primary routes of human exposure to heavy metals: oral and inhalation. In oral exposure, the main source is plant-based food, which accounts for 40–80% of heavy metal intake; atmospheric air and water contribute 20–40% of HMs. The second route is inhalation exposure, although it is less common [14].

According to the decision of the UN Economic Commission for Europe[3], the group of the most hazardous (and thus prioritized for monitoring, control, and regulation) HMs includes mercury, lead, cadmium, chromium, manganese, nickel, cobalt, vanadium, copper, iron, zinc, and antimony (as well as arsenic and selenium) [15].

Poisoning with HM salts occurs through ingestion into the digestive system, inhalation of vapors, or exposure via mucous membranes and skin. Multiple systems and organs — the CNS, kidneys, intestines, liver, endocrine organs, heart, and blood vessels — are affected. HM salts are capable of accumulating in the body, circulating for extended periods and gradually releasing into the bloodstream from their depots, rendering the process chronic [16].

Overall, the mechanisms of HM toxicity have been studied in detail. The impact of HM salts on the body is determined by numerous factors, including the nature of the metal, the type of compound, and its concentration. Metal ions are part of coenzymes. One of the primary mechanisms of HM toxicity consists in their competition with essential metals for binding sites in proteins. Furthermore, many protein macromolecules contain free sulfhydryl groups that can interact with ions of toxic metals, such as cadmium, lead, mercury, etc. This interaction subsequently leads to the loss of protein function and the development of toxic effects [11].

Lead poisoning (Pb). According to estimates presented in the updated release of the WHO publication "The impact of chemicals on public health: known and unknown for 2021," nearly half of the two million deaths caused by chemical substances in 2019 were due to lead exposure. Globally, the long-term health consequences of lead exposure through various routes of entry (oral, inhalation, transcutaneous) result in the loss of 21.7 million years of life due to disability and death (disability-adjusted life years, DALYs). Lead, as a contributing factor to disease development, accounts for up to 30% of all idiopathic intellectual disabilities, 4.6% of cardiovascular diseases, and approximately 3% of chronic kidney diseases.[4]

Lead poisoning, in the setting of oxidative stress, results in leukocytosis, lymphocytosis, a decrease in total protein, albumin, and globulin levels [17]. The genotoxic effect of lead was demonstrated experimentally by administering lead acetate in water (Pb) intraperitoneally to laboratory animals in small doses over six weeks. By the end of the observation period, the animals had developed moderate subchronic intoxication with signs characteristic of lead effects (caused by impaired heme formation and increased synthesis of cytochrome P450 in the liver) [18].

It was found that approximately 80% of lead entering the body accumulates in bone tissue, while the remaining 20% is distributed in adipose tissue, kidneys, and liver, binding to sulfhydryl groups of proteins and forming toxic compounds [11, 19, 20]. The primary target organs in lead poisoning are the hematopoietic system, CNS, and the kidneys. Lead poisoning is accompanied by changes in antioxidant status, ionic mimicry as a mechanism of molecular lead toxicity, alterations in the structure and function of intracellular organelles in neuronal cells, induction of autophagy through the PI3K/AKT/mTOR signaling pathway, effects on the cellular receptor apparatus, changes in synaptic plasticity, and impacts on the cellular genetic apparatus [21]. In the brain, diffuse edema of gray and white matter can be observed, as well as dystrophic changes in cortical and ganglionic neurons and demyelination of white matter [12].

The angiotoxic effect of lead is manifested through activation of mitogen-activated protein kinase signaling pathways, which triggers a cascade of reactions for the synthesis of pro-inflammatory proteins, leading to increased vascular resistance and blood pressure [22].

Cadmium poisoning (Cd). According to the WHO, cadmium ranks fifth among the most hazardous chemical substances affecting the human body. This metal is typically present in the environment in small quantities. However, due to human activities, cadmium levels are

[3] Protocol on Heavy Metals. UNECE; 1998.
[4] World Health Organization. URL: https://www.who.int/ru/news-room/fact-sheets/detail/lead-poisoning-and-health (access date: 05.04.2025).

gradually increasing each year [23]. Both in Russia and globally, the primary area of cadmium consumption is the production of nickel-cadmium batteries. Other applications of cadmium include anti-corrosion coatings, pigments, polyvinyl chloride stabilizers, and semiconductors for solar cells [24].

Cadmium can enter the human body through inhalation, via cigarette smoke (cadmium accumulates in tobacco leaves) or the air with accumulated road dust particles, including that generated from tire and brake pad wear. Orally, cadmium is ingested via its accumulating foods, such as mushrooms, various plants, and meat (pork or beef) [25]. Additionally, cadmium can also enter food products through packaging (typical of canned goods, particularly those made of materials prohibited for contact with food) [26]. Cadmium is chemically similar to zinc, capable of its replacement in biochemical reactions, acting as a pseudo-inducer or pseudo-inhibitor of zinc-containing enzymes [27].

The pathogenesis of the toxic effects of HMs on the body exhibits uniform features characteristic of many types of damage: activation of lipid peroxidation (LPO) and damaging effects on intracellular proteins [28]. Since liver cells do not absorb the cadmium-protein complex, it is transported from the gastrointestinal tract directly to the kidneys [10]. The most pronounced inhibitory effect of Cd is on the antioxidant system, leading to oxidative cell damage [29].

Cadmium poisoning is accompanied by disruptions in protein synthesis and nucleic acid metabolism. Cadmium also possesses carcinogenic and mutagenic properties; thus, experiments have confirmed its teratogenic effect, which is associated with cell damage during early stages of organogenesis [30].

Chronic cadmium intoxication leads to impaired functional state of the kidneys, characterized by significant changes in glomerular filtration rate and tubular water reabsorption. The nephrotoxic effect of cadmium alters electrolyte metabolism, marked by increased excretion of sodium and calcium and decreased excretion of potassium. In a situation of chronic cadmium poisoning, the concentrating function of the kidneys is impaired [31].

Arsenic poisoning (As). Arsenic, a natural component of more than 200 natural minerals, is included in the WHO list of 10 chemical elements that pose significant public health threats[5].

The mechanism of action of trivalent and pentavalent arsenic compounds differs. Trivalent arsenic acts by blocking the pyruvate dehydrogenase complex, which plays a key role in glycolytic processes. Trivalent arsenic reduces ATP resynthesis and the formation of oxaloacetic acid from pyruvate (disrupting pyruvate gluconeogenesis), ultimately leading to hypoglycemia. Trivalent arsenic also inhibits the activity of glutathione synthase, glucose-6-phosphate dehydrogenase, and glutathione reductase, resulting in glutathione deficiency in the liver and impaired arsenic detoxification processes. Due to disrupted glycolysis, acetylcholine synthesis is also impaired, which is a cause of peripheral neuropathy [32].

When entering the human body orally (through food or water) and via inhalation (atmospheric air, dust) in elevated quantities, arsenic can primarily cause liver dysfunction, allergic reactions, skin changes (hyperkeratosis, dermatitis), vascular damage (often in the lower extremities), hearing loss, immunosuppression, impaired hematopoiesis, and severe neurological disorders (increased CNS excitability, irritability, headaches). Chronic As intoxications lead to damage to peripheral nerve fibers, where demyelination is severely pronounced, even to the point of axonal destruction. Dermatological manifestations include the appearance of dark brown pigmentation in the form of isolated or merging spots on the skin, hyperkeratosis of the palms and soles, followed by the development of epidermoid carcinomas in these areas [33].

The main complications of acute As intoxication are the development of intravascular hemolysis, acute renal and hepatic failure, and cardiogenic shock. Long-term consequences of acute poisonings in children may include significant hearing loss. Damage to the nervous system manifests as toxic encephalopathy (impaired speech, coordination, epileptiform seizures, psychoses) [12].

Thallium poisoning (Tl). In the overall structure of HM poisonings, thallium compounds rank relatively low; however, the severity of their course, complex differential diagnosis, and challenges in treatment necessitate special attention to these intoxications[6].

A significant role in the mechanism of thallium toxic action is played by blocking of sulfhydryl groups and suppression of thiol-dependent enzymes that regulate mitochondrial permeability, leading to water influx and swelling. Thallium was established to disrupt glutathione metabolism, enhance lipid peroxidation processes, damags the membrane apparatus, and cause cell death [34, 35]. Thallium also delays protein synthesis by acting on ribosomes (particularly the 60S subunit), leading to impaired keratinization and alopecia. Thallium intoxication results in disrupted riboflavin metabolism by forming

[5] World Health Organization. URL: https://www.who.int/ru/news-room/fact-sheets/detail/lead-poisoning-and-health (access date: 05.04.2025).
[6] Livanov GA. Poisoning with Thallium Compounds (Clinical Presentation, Diagnosis and Treatment). A Guide for Physicians. Saint Petersburg; 2016.

an insoluble complex with riboflavin and leading to riboflavin deficiency and impaired cellular energy supply [36, 37]. Certain symptoms of thallium poisoning, such as peripheral neuropathy, alopecia, and dermatitis, confirm riboflavin deficiency (as demonstrated in animal models of riboflavin deficiency). All this leads to the blocking of active transport of alkali metal ions and causes disruptions in various functional systems, which accounts for diverse clinical patterns [38].

Given the above, one of the most potent and widespread forms of chemical pollution is HM contamination [10]. Lead is the most hazardous HM to human health, due to its ability to replace metal ions essential for biochemical processes and disrupt biological processes in cells [39]. It is important to note that the kinetics of such HMs as lead and cadmium are similar. Indeed, they lack their own transporters and enter the cells and blood using transport systems intended for normally present metals and trace elements. However, although the negative impact of cadmium on human health is beyond doubt, the relationship between the dose levels of cadmium intake and deterministic genotoxic effects remain to be understood. Such effects are likely to be related to the multifactorial nature of cadmium action resulting from cadmium ions binding to numerous cellular targets. Consequently, the development of approaches to reducing cadmium-induced toxicity is a priority direction in toxicological research [24].

Poisoning by medicinal neurotropic drugs

According to data from the World Association of Toxicological Centers (International Association of Forensic Toxicologists, TIAFT), acute poisonings with neurotropic drugs rank among the top positions in the structure of poisoning accidents [40]. Poisoning with barbiturates account for approximately 20–25% of all cases of acute poisonings for which patients are admitted to specialized toxicological hospitals [41].

Barbiturate poisoning. Recent years have seen a decline in barbiturate poisoning cases [42], attributed to both the reduced scope of application and distribution of barbituric acid derivatives, as well as the introduction of more modern/safer drugs, including benzodiazepines. Despite this, barbiturates remain among the top 15 classes of medications causing patient deaths from drug intake [43].

According to data from the Department of Clinical Toxicology of the Dzhanelidze Research Institute of Emergency Care, in 2015, out of 286 cases of barbiturate poisoning, 104 patients (36.3%) were over 60 years old, of whom 11 (3.8% of the elderly group) died. In 2016, out of 482 patients, 123 (25.5%) were of elderly and senile age, with 21 (17.1% of the elderly group) experiencing a fatal outcome. Poisoning by barbituric acid derivatives is most commonly provoked by the use of combined medications such as Corvalol or Valocordin®, which, in addition to phenobarbital, contain ethanol, ethyl ester of α-bromoisovaleric acid, and peppermint leaf oil. The severity of the patients' condition was determined by the development of toxic encephalopathy, clinically manifested by consciousness disorders of varying severity: from mild stupor to atonic coma [44].

Yesin et al. indicate that barbiturates are most frequently used in suicide attempts by individuals over 60 years old (27.6% of all suicidal poisonings in this age category), whereas among persons aged 18–59, suicidal barbiturate poisonings account for 11.8% [45].

For humans, a single oral intake of approximately 10 therapeutic doses of the drug is considered lethal. The lethal dose of phenobarbital ranges 2–10 g [46]. In high doses, barbiturates exert a depressant effect on the sensory areas of the cerebral cortex, thereby reducing motor activity and suppressing cerebral functions [42].

Clinically pronounced symptoms of toxic barbiturate poisoning may manifest only several hours after drug ingestion. Intoxication presents as nystagmus, ataxia, dizziness, headache, increasing psychomotor retardation, suppression or complete loss of reflexes, marked drowsiness or agitation, progressing to respiratory depression, pupillary constriction (alternating with paralytic dilation), increased or decreased heart rate while maintaining normal cardiac rhythm, and cyanosis. Pulmonary edema and coma development are among the complicated symptoms of barbiturate intoxication [46].

A detailed study of the clinical course of barbiturate intoxication conducted by Alexandrovsky et al. identified the main syndromes in 385 examined patients. These were comatose state and other neurological disorders (pupillary constriction, sensory disturbances, increased or decreased tendon and skin reflexes, etc.), respiratory and hemodynamic impairments [47].

Post-hypoxic brain injury is one of the most serious consequences of barbiturate poisoning. This complication is observed in 35% of patients with severe and extremely severe poisoning, manifested as reduced cognitive function, paralysis, paresis, and impaired functioning of internal organs. In some cases, the development of cerebral edema can lead to fatal outcomes or irreversible damage to the CNS [22].

Intoxication with organic solvents

Methanol poisoning. Methanol, also known as methyl or wood alcohol, is an organic chemical compound and the simplest aliphatic alcohol with the chemical formula of CH_3OH. It is a colorless, poisonous liquid widely used in the industrial sector as a solvent, in the synthesis of organic compounds, in the production of resins and dyes, and is a component of windshield washing fluids. Due to the active search for alternative energy sources and the consideration of alcohols as a replacement for hydrocarbon fuels, the use of methanol is forecasted to increase worldwide [48].

The problem of methanol poisoning is extremely acute. According to Rosstat data, poisoning with alcohol-containing products continues to be a leading cause of fatal outcomes due to chemical poisoning. The most tragic case of mass methanol poisoning occurred in Irkutsk (December 2016), where 77 people died, with the maximum number of fatalities in the working-age population [49]. Another highly publicized mass methanol poisoning in Russia occurred during the period from June 3 to 5, 2023, when residents of several regions consumed alcoholic products under the brand of Mr. Cider. As a result, 47 people died and at least 106 were affected. Loskutnikova et al., Yakovenko et al. have also noted a wave-like dynamics in mortality rates from methanol poisoning [50, 51].

According to the statistical data presented in the state report "On the State of Sanitary and Epidemiological Well-Being of the Population in the Russian Federation in 2021," 470,358 cases of acute poisoning with alcohol-containing products were registered in the Russian Federation in 2012–2021, with 124,813 of them being fatal (26.5%). The rate of acute poisoning with alcohol-containing products in 2021 was 21.19 cases per 100,000 population.[7]

Thus, the statistical data on poisonings in the Vologda Oblast shows that methanol poisoning consistently ranks second after the cases of ethanol poisoning (Table 2).

Acute methanol poisoning progresses through four stages: initial stage, stage of latent manifestations or false well-being, stage of pronounced clinical manifestations, and the period of poisoning consequences.[8]

The initial stage, lasting 1–12 h, is characterized by manifestations of ordinary alcohol intoxication caused by the narcotic effect of this alcohol on the CNS, with the degree of intoxication being less pronounced than that with comparable doses of ethyl alcohol[9]. The stage of false well-being follows the period of intoxication [53].

The stage of pronounced clinical manifestations is characterized by the development of toxic gastritis, toxic encephalopathy with headaches and dizziness, psychomotor agitation, stupor and confusion, loss of contact. At the culminating stage, symptoms of severe general

Table 2. Number of alcohol poisoning accidents in 2020–2022 (per 100,000 population) in the Vologda Oblast according to data from the Local Territorial Center for Hygiene and Epidemiology

Parameter	2020		2021		2022	
	total	of which fatal	total	of which fatal	total	of which fatal
Toxic effects of alcohol (T51) — total	6.4	3.7	4.7	1.8	4.5	1.4
Ethanol (T51.0)	4.4	2.7	3.5	1.4	3.2	0.8
Methanol (T51.1)	0.1	0.1	0.1	0.0	0.4	0.4
2-propanol (T51.2)	0.1	0.1	0.3	0.3	0.1	0.1
Fusel oil (T51.3)	0.2	0.2	0.0	0.0	0.0	0.0
Other alcohols (T51.8)	0.0	0.0	0.1	0.1	0.0	0.0
Unspecified alcohol (T51.9)	1.6	0.7	0.8	0.1	0.7	0.1

Table prepared by the authors according to the source [52]

[7] On the State of Sanitary and Epidemiological Well-Being of the Population in the Russian Federation in 2021: State Report. M.: Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing; 2022.

[8] Analysis of acute chemical etiology poisonings, including alcohol-related fatal cases for 2022. URL: https://fbuz35.ru/files.aspx?id=4038db6b560c4057b9be4e4358af0412 (access data: 10.04.2025).

[9] Clinical Guidelines "Toxic Effects of Methanol and Ethylene Glycol" (approved by the Ministry of Health of Russia). https://legalacts.ru/doc/klinicheskie-rekomendatsii-toksicheskoe-deistvie-metanola-i-etilenglikolja-odobreny-minzdravom/?ysclid=mdfwi7hjv1220621463 (access data: 10.04.2025).

intoxication develop in the form of acute respiratory and cardiovascular failure. Ophthalmological disorders are among the main consequences of methanol poisoning (in surviving patients). The main complications of methanol poisoning include toxic kidney damage, acute toxic myocarditis, and, in the long-term period, psychoneurological disorders in the form of memory impairment, emotional lability, disorders such as "body schema disturbance".

A characteristic feature of methanol intoxication consists in its metabolism occuring according to the principle of "lethal" synthesis, resulting in the formation of more poisonous substances: formaldehyde, whose toxicity is 33 times higher than that of methanol itself, and formic acid. Part of the formaldehyde interacts with proteins, and another part is converted into formic acid. The metabolism of formaldehyde occurs through the two main ways: with the help of tetrahydrofolic acid and reduced glutathione. Formic acid is rapidly formed during the oxidation of formaldehyde, and its further metabolism proceeds slowly. The main metabolic transformations occur in the liver tissue, which has the greatest ability to oxidize formaldehyde. In addition, a significant part of methanol is excreted through the lungs.

Poisoning with diols (1,4-butanediol and related compounds). Among the most frequently encountered poisoning exposures to organic solvents, 1,4-butanediol (1,4-BD) holds a special place. It is a dihydric aliphatic alcohol widely used in industrial sectors as a solvent, an intermediate in organic synthesis, and the production of plastics[10]. In recent years, this compound has increasingly been used illegally to achieve a specific emotional state (euphoria). Thus, the prevalence of 1,4-butanediol use as a psychoactive substance (PAS) among the drug-dependent population ranges 22.3–43.7%; moreover, it is often consumed concurrently with other PAS (ethanol, amphetamine and its derivatives, etc.) [54, 55].

The toxicodynamics of oral 1,4-BD use includes rapid absorption by the stomach and upper small intestine into the blood from and passage through the blood–brain barrier. After oxidation by alcohol dehydrogenase (ADH) to gamma-hydroxybutyraldehyde, it is metabolized to GHB and its carboxyl metabolite (glucuronide) followed by metabolization in the liver to aldehyde metabolites under the influence of ADH [56]. Moreover, 1,4-butanediol is metabolized to GHB, on average, within 1 min [57]. At the same time, studies on volunteers have shown significant individual differences in the rate of metabolism of 1,4-butanediol to GHB, presumably associated with varying degrees of alcohol dehydrogenase activity [58]. The minimum toxic dose of 1,4-BD for humans is 5–20 g (88–300 mg/kg) [59].

The clinical pattern of acute 1,4-butanediol poisoning manifests as behavioral disorders in the form of psychomotor agitation accompanied by anxiety and aggression, hallucinations, disorientation, and delirium [59]. In severe 1,4-BD poisoning, loss of consciousness up to coma, neurological disorders, impaired respiratory function, cardiovascular activity, and various metabolic disturbances are observed [55].

A number of studies have presented illustrative clinical cases of acute oral poisonings with 1,4-BD. For instance, Sinchenko et al. described a clinical case of acute 1,4-BD poisoning combined with ethanol in a young man who had long used this psychoactive substance in small doses to enhance sexual arousal and physical endurance. Acute poisoning occurred after a single intake of an excessive dose of the psychoactive substance, leading to the development of a convulsive syndrome, depression of consciousness to the level of stage II coma, and the onset of toxic encephalopathy [55]. Livanov et al. described a clinical case of severe acute oral poisoning with 1,4-butanediol in a 12-year-old adolescent girl, accompanied by toxic-hypoxic encephalopathy and stage III coma, resulting from the accidental ingestion of 1,4-BD [59].

In the USA and EU, the issue of the narcotic use of gamma-hydroxybutyric acid (GHB) and its precursors (1,4-butanediol and gamma-butyrolactone) is related to the frequent use of these substances in the commission of sexual crimes [57].

Industrial chemicals and toxic compounds

Carbamate poisoning. Carbamates are derivatives of carbamic acid used in agriculture as insecticides and pesticides, as well as in pharmacology as medicinal products (tranquilizers, muscle relaxants, antidotes, etc.). The main routes of carbamate entry into the human body are the respiratory organs and intact skin, as well as the gastrointestinal tract[11].

Carbamate intoxication holds a significant position in the structure of poisoning caused by neurotoxic chemicals. These compounds can cause dysfunction of the cholinergic system by activating nicotinic and muscarinic receptors. A number of carbamic acid derivatives are highly toxic compounds, reversible (unlike organophosphates — OPs) inhibitors of cholinesterases, leading to the so-called "cholinergic crisis." This condition is associated with the development of generalized convulsive syndrome, which results in coma and

[10] Great Russian Encyclopedia: Scientific and Educational Portal. https://bigenc.ru/c/1-4-butandiol-b997fe/?v=8031024 (access data: 05.04.2025).
[11] Gerunova L, Boyko T. Toksikologiya pestitsidov. Moscow: ID Nauchnaya biblioteka; 2021.

death in severe cases. Due to the rapid hydrolysis of the C=O bond (decarbamoylation of the enzyme) during acute carbamate intoxication, cholinesterase activity in survivors recovers within a few hours, with complete restoration of cholinesterase function observed within 24–48 h [60].

The clinical pattern of carbamate intoxication is determined by the accumulation of acetylcholinesterase at nerve endings. The symptoms of intoxication can be classified into the following groups [61]:

1. Muscarinic-like manifestations: increased bronchial secretion, profuse sweating and salivation, lacrimation, pupillary constriction, bronchospasm, abdominal cramps (vomiting and diarrhea), bradycardia.

2. Nicotinic-like manifestations: fasciculations of small muscles (in severe cases, also respiratory and diaphragmatic muscles), tachycardia.

3. Symptoms and signs of CNS damage: headache, dizziness, anxiety, memory loss, convulsions, coma.

4. Depression of the respiratory center.

All these symptoms manifest in various combinations and may vary in presentation and sequence depending on the substance, dose, and route of exposure. The duration of symptoms is generally shorter than with OP exposure [62]. In severe cases of poisoning, death may occur due to asphyxia from muscle spasm or acute heart failure [63]. Long-term consequences include significant impairments in cognitive and behavioral abilities [61].

Based on the analysis of the social status of individuals poisoned by carbamates, Reddy et al. revealed unexpected results: 23% of the victims were farmers, 27% were temporary workers, 21% were homemakers, 11% were permanent employees, and 8% were students [64]. Thus, in addition to the risk of poisoning in workplaces with hazardous conditions or during various terrorist incidents, accidental or intentional self-poisoning, as well as toxic effects from drug overdoses, are quite widespread [65]. According to the WHO, approximately one million cases of unintentional acute pesticide poisoning are registered worldwide each year.

CONCLUSION

Our study has reviewed the characteristics of the main representatives of neurotropic toxicant groups that pose threat to public health. It was revealed that although the central nervous system is the primary target organ for neurotropic toxicants, their toxicity adversely affects other organs. This explains the wide spectrum of pathologies developing as a result of exposure to these poisons (impairments of external respiratory function, cardiovascular activity, various metabolic disturbances up to multiorgan pathology). Signs of toxic action can manifest both immediately after intoxication and in the period of long-term consequences.

The health risk of poisonings by neurotropic toxicants is associated with the following aspects. First, such poisoning can occur not only in industrial but also in domestic settings. Second, poisoning by neurotropic toxicants leads to the development of chronic pathological processes in the victim's body. In addition, acute poisoning with some neurotropic toxicants includes a latent period lasting up to several days, which complicates the diagnosis and hinders the timely initiation of treatment. Third, although the main disorders in the acute period, manifested in the nervous, respiratory, and cardiovascular systems, can be managed through drug therapy, there is a risk of developing long-term consequences.

Future studies should address the formation mechanisms of long-term consequences in cases of intoxication with neurotropic toxicants, as well as the search for preventive and therapeutical measures to combat the arising pathological conditions.

References

1. Grigoriev IM, Voronko EA, Ostapenko EN. Organization of medical care in chemical accidents and emergencies. *Emergency Medicine*. 2013;4:27–41 (In Russ.). EDN: RPEWUL
2. Kobyakova OS, Shibalkov IP, Solomatnikov IA, Timonin SA, Shchur AE, Lagutin MD, et al. Medical and demographic situation in Russia: long-term trends, forecasts, and reserves for improvement. *Health Risk Analysis*. 2024;2:4–17 (In Russ.). https://doi.org/10.21668/health.risk/2024.2.01
3. Solonin SA, Belova MV, Tereshkina NE, Kasholkina EA, Tyurin IA, Godkov MA, et al. The structure of acute poisoning of chemical etiology in a metropolis against the background of the COVID-19 pandemic in 2020–2021. *Extreme Medicine*. 2023;25(4):174–83 (In Russ.). https://doi.org/10.47183/mes.2023.052
4. Vasiliev SA, Livanov GA, Batotsyrenov BV. Features of the organization of resuscitation care for patients in critical condition with acute poisoning. *Medical Alphabet*. 2013;2(14):45–9 (In Russ.). EDN: RMXMYL
5. Goldfarb YuS, Badalyan AV, Gerasimenko MYu, Shchetkin VA, Potskhveriya MM. Rehabilitation program for acute poisoning in a toxicological hospital. *Russian Journal of Physiotherapy, Balneology and Rehabilitation*. 2021;20(2):99–117 (In Russ.).

https://doi.org/10.17816/1681-3456-2021-20-2-2
6. Kutsenko SA. *Fundamentals of Toxicology*. St. Petersburg: Medicine; 2004 (In Russ.).
EDN: QKMWIB
7. Belonogov IA, Samokhin DA. *Toxicology and medical protection*. Minsk: Higher School; 2014 (In Russ.).
EDN: VRRYIR
8. Velikorodnaya YuI, Mamulaishvili NI, Pocheptsov AYa. Neurotoxic effects in the central nervous system in chronic organophosphorus intoxication (experimental study). *Journal of Volgograd State Medical University*. 2013;3(47):56–61 (In Russ.).
9. Shustov EB, Melnikova MV, Zorina VN, Melekhova AS, Belskaya AV, Bondarenko AA, et al. Theoretical aspects of the interaction of chemical and physical adverse effects in conditions of combined environmental pollution. *Bulletin of Education and Development of Science of the Russian Academy of Natural Sciences*. 2022;4:83–94 (In Russ.).
https://doi.org/10.26163/RAEN.2022.50.76.011
10. Akhpolova VO, Brin VB. Actual concepts of heavy metals' kinetics and pathogenesis of toxicity (literature review). *Journal of New Medical Technologies*. 2020;27(1):55–61 (In Russ.).
https://doi.org/10.24411/1609-2163-2020-16578
11. Galenko MS, Gravel IV, Velts NY, Alyautdin RN. Standardization of the content of heavy metals and arsenic as a safety factor in the use of herbal medicines. *Safety and Risk of Pharmacotherapy*. 2021;9(2):61–8 (In Russ.).
https://doi.org/10.30895/2312-7821-2021-9-2-61-68
12. Teplaya GA. Heavy metals as a factor of environmental pollution (literature review). *Astrakhan Bulletin of Environmental Education*. 2013;1(23):182–92 (In Russ.).
EDN: PXNTRR
13. Elizarova DI, Ivanushkina TS. Assessment of the impact of solid waste landfills on the environment. *Scientific Journal of Young Scientists*. 2023;4(34):12–6 (In Russ.).
EDN: HSWBSY
14. Kutlin NG, Agliullina KS, Safina GR, Abdulvaleev AM. Monitoring milk for heavy metal content. *Ways to implement the Federal Scientific and Technical Program for the Development of Agriculture for 2017–2025*. Proceedings of the International Scientific and Practical Conference dedicated to the 75th Anniversary of the Kurgan Region. Lesnikovo: 2018 (In Russ.).
EDN: UOTXHG
15. Tsyplikhin NO, Komarova EV, Fedorova MG. Morphological features of the thyroid gland under the influence of nitrate compounds and heavy metals: a meta-analysis of experimental studies. *Bulletin of Penza State University*. 2024;3(47):22–5 (In Russ.).
EDN: FBMQDO
16. Tunakova YuA, Novikova SV, Valiev VS, Fayzullin RI. Method for quantitative assessment of metal accumulation in the human body under polymetallic pollution of urban ecosystem components. *Scientific Notes of Kazan University. Natural Sciences Series*. 2024;166(2):266–82 (In Russ.).
https://doi.org/10.26907/2542-064X.2024.2.266-282
17. Abdelhamid FM, Mahgoub HA, Ateya AI. Ameliorative effect of curcumin against lead acetate-induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. *Environmental Science and Pollution Research*. 2020;1:10950–65.
https://doi.org/10.1007/s11356-020-07718-3
18. Minigalieva IA, Shtin TN, Makeyev OH, Panov VG, Privalova LI, Gurvic VB, et al. Some outcomes and a hypothetical mechanism of combined leadand benzo(a)pyrene intoxication, and its alleviation with a complex of bioprotectors. *Toxicology Reports*. 2020;7:986–94.
https://doi.org/10.1016/j.toxrep.2020.08.004
19. Pratush A, Kumar A, Hu Z. Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. *International Microbiology*. 2018;21:97–106.
https://doi.org/10.1007/s10123-018-0012-3
20. Yang CM, Chien MY, Chao PC, Huang CM, Chen CH. Investigation of toxic heavy metals content and estimation of potential health risks in Chinese herbal medicine. *Journal of Hazardous Materials*. 2021;412:125–42.
https://doi.org/10.1016/j.jhazmat.2021.125142
21. Sutunkova MP, Nikogosyan KM, Ryabova YuV, Keskevich AA, Minigalieva IA, Butakova IV, et al. Current understanding of the mechanisms of lead toxic effects on the central nervous system (literature review). *Occupational Medicine and Human Ecology*. 2023.4:196–215 (In Russ.).
https://doi.org/10.24412/2411-3794-2023-104115
22. Dzugkoev SG, Dzugkoeva FS, Margieva OI. Analysis of the mechanisms of lead toxicity and their pathogenetic correction. *Sechenov' Russian Physiological Journal*. 2022;108(5):626–35 (In Russ.).
EDN: HKRPRI
23. Ivut AI. Environmental pollution with cadmium as an ecological health risk. *International Student Scientific Bulletin*. 2021;2:1–12 (In Russ.).
EDN: ZMSAFX
24. Fazlieva AS, Daukaev RA, Karimov DO. Influence of cadmium on population health and methods for preventing its toxic effects. *Occupational Medicine and Human Ecology*. 2022;1:220–35 (In Russ.).
EDN: GLPABT
25. Gusarova ML, Baranovich ES, Volkova NI, Chelysheva ON, Zyuzin IE. Radioecological analysis of safety indicators of livestock products. *The Veterinarian*. 2024;4:49–57 (In Russ.).
26. Mikhovich AP. Lead and cadmium as dangerous heavy metals, their main sources, migration routes and impact on the human body. *Current issues of radiation and environmental medicine, radiation diagnostics and radiation therapy*. Collection of materials of the 1st inter-university scientific and practical Internet conference of students, master's students, postgraduates and young scientists. 2017 (In Russ.).
EDN: YGQQNK
27. Oberens D, Harland B, Skalny A. *Biological role of macro- and microelements in humans and animals*. St. Petersburg: Nauka; 2008 (In Russ.).
28. Genchi G, Sinikropi MS, Lauria G, Carocci A, Catalano A. The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*. 2020;7(11):1–24.
https://doi.org/10.3390/ijerph17113782

29. Rafati-Rahimzadeh M, Rafati-Rahimzadeh M, Kazemi S, Moghadamnia A. Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*. 2017;8(3):135–45. https://doi.org/10.22088/cjim.8.3.135
30. Kuzmin SV, Rusakov VN, Setko AG, Sinitsyna OO. Toxicological and hygienic aspects of the dietary cadmium intake and its human effects: a literature review. *Public Health and Life Environment — PH&LE*. 2024;32(7):49–57 (In Russ.).
31. Usmanova EN, Fazlieva AS, Karimov DO, Khusnutdinova NYu, Repina EF, Daukaev RA. Dynamics of cadmium accumulation in the liver and kidney of rats with acute intoxication. *Occupational Medicine and Human Ecology*. 2019;2(18):69–74 (In Russ.). EDN: EFDLLB
32. Satapathy D, Dutta TK, Chatterjee A, Yadav SK, Dutta S, Das AM, et al. Ameliorating arsenic toxicity in young goats: assessing vitamin E and Saccharomyces cerevisiae on feed intake, carcass quality, mineral profiles in tissues and impending health risks to humans. *Environmental Geochemistry and Health*. 2025;47(5):1–31. https://doi.org/10.1007/s10653-025-02439-3
33. Bakanov KB, Bakhareva AA, Fadeeva YuA, Gonostareva DA, Yugova TS. Toxicological aspects of arsenic use in medical practice. *Youth, Science, Medicine*. Proceedings of the 64th All-Russian Interuniversity Student Scientific Conference with International Participation. 2018 (In Russ.). EDN: YRHPUD
34. Zavaliy LB, Simonova AYu, Potskhveria MM, Ostapenko YuN, Gadzhieva MG, Petrikov SS. Diagnosis and treatment of poisoning with thallium. *Toxicological Review*. 2018;5:9–15 (In Russ.). https://doi.org/10.36946/0869-7922-2018-5-9-15
35. Xu H, Luo Y, Wang P, Zhu J, Yang Z, Liu Z. Removal of thallium in water/wastewater: A review. *Water Research*. 2019;165:149–81. https://doi.org/10.1016/j.watres.2019.114981
36. Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. Thallium use, toxicity, and detoxification therapy: an overview. *Applied Sciences*. 2021;11(18):1–15. https://doi.org/10.3390/app11188322
37. Molina CP, Verstraeten SV. Thallium (III)-mediated changes in membrane physical properties and lipid oxidation affect cardiolipin-cytochrome *c* interactions. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 2008;1778(10):2157–64. https://doi.org/10.1016/j.bbamem.2008.04.013
38. Baj J, Forma A, Kobak J, Tyczyńska M, Dudek I, Maani A, et al. Toxic and Nutritional Optic Neuropathies — An Updated Mini-Review. *International Journal of Environmental Research and Public Health.* 2022;19(5):3092. https://doi.org/10.3390/ijerph19053092
39. Titov AF, Kaznina NM, Karapetyan TA, Dorshakova NV. Lead as a contaminant for living things. *Journal of General Biology*. 2020;81(2):147–60 (In Russ.). https://doi.org/10.31857/S0044459620020086
40. Bebyakina EE, Yatsinyuk BB, Akhmedova EG, Volkova NA. Peculiarities of the epidemiology and course of clinical manifestations of acute poisoning with neurotropic drugs (according to the data of the BUKh «District Clinical Hospital»). *Healthcare of Yugra: Experience and Innovations*. 2024;4(41):70–87 (In Russ.).
41. Tropskaya NS, Kislyakova EA, Vilkova IG, Gurman YuV, Kislitsyna OS, Zherebtsov AV, et al. Disturbances in the functional state of the central nervous system, intestinal peristalsis, and microbiocenosis in experimental barbiturate poisoning. *Journal Biomed*. 2022;18(3):45–9 (In Russ.). https://doi.org/10.33647/2074-5982-18-3-45-49
42. Dimitrova S, Dragomanova S, Kehayova G. Intravenous lipid emulsions in anticonvulsants' toxicity. *Scientia Pharmaceutica*. 2024;92(3):31–7. https://doi.org/10.20944/preprints202404.1698.v1
43. Greene SC, Wyatt K, Cates AL, Weiss S. Anticonvulsant fatalities reported to the American Association of Poison Control Centers 2000–2019. *Seizure*. 2023;106:1–6. https://doi.org/10.1016/j.seizure.2023.01.010
44. Livanov GA, Lodyagin AN, Kovalenko AL, Batotsyrenov BV, Glushkov SI, Prokopovich GA, et al. Peculiarities of the clinical picture of acute poisoning with barbituric acid derivatives and their therapy in elderly and senile patients. *Advances in Gerontology*. 2018;31(2):239–45 (In Russ.).
45. Esin GV, Livanov PA, Soldatova VYu, Livanov AS, Picovskiy VYu. Toxic effects of barbiturates. *Medical Alphabet*. 2015;1(3):25–30 (In Russ.). EDN: UNDPEJ
46. Crellin SJ, Katz KD. Pentobarbital toxicity after self-administration of Euthasol veterinary euthanasia medication. *Case Reports in Emergency Medicine*. 2016;1–4. https://doi.org/10.1155/2016/6270491
47. Aleksandrovsky VN, Kareva MV, Rozhkov PG. Acute poisoning with barbituric acid derivatives. Barbituric coma. *Toxicological Review*. 2022;30(6):359–69 (In Russ.). https://doi.org/10.47470/0869-7922-2022-30-6-359-369
48. Kholikova SD, Ismatullaev HM, Ismailova LA. Prospective ways of application of methanol. *Universum: Chemistry and Biology*. 2021;5(83):22–6 (In Russ.). https://doi.org/10.32743/UniChem.2021.83.5.11632
49. Zimina LA, Zhogolev PV, Semyonov AV, Lelyuh TD, Lunyonok SV, Maslauskaite LS, et al. Analysis of lethal cases of mass methyl alcohol poisining in Irkutsk. *Sciences of Europe*. 2018;32:28–35 (In Russ.).
50. Loskutnikova EI, Gil AYu, Alekhin IN, Khalfin RA. Analysis of morbidity with poisonings with ethanol and surrogate alcohols in irkutsk region between 2010 and 2017. *Sechenov Medical Journal*. 2019;10(2):36–44 (In Russ.). https://doi.org/10.47093/22187332.2019.2.36-44
51. Yakovenko TA, Onan OYa, Doluma AM, Tarasov AYu. Acute methanol poisoning: a literature review. *Magister Journal*. 2022;12–1(135):4–8 (In Russ.). EDN: JGDQPM
52. Popova EA, Lyubchenko AA, Shivrina TG, Rakhmanova EA, Khabarova OI, Bolshakova MA, et al. Clinical and laboratory parameters, characteristics and results of treatment of patients with methyl alcohol poisoning. *Bulletin of Khakass*

State University named after N.F. Katanov. 2017;19:126–9 (In Russ.).
EDN: ZVKVZR

53. Kuznetsov OA, Fedorov AV. Acute methanol poisoning with a favorable outcome (series of clinical observations). *Narcology*. 2019;4:64–7 (In Russ.).
EDN: ZIOPTF

54. Sinenchenko AG, Batotsyrenov ChB, Lodyagin AN, Sinenchenko GI, Kovalenko AL. Delirium in acute poisoning with 1,4-butanediol and its correction. *General Reanimatology*. 2021;17(6):42–8 (In Russ.).
https://doi.org/10.15360/1813-9779-2021-6-42-48

55. Miró Ò, Galicia M, Dargan P, Dines AM, Giraudon I, Heyerdahl F, et al. Intoxication by gamma hydroxybutyrate and related analogues: Clinical characteristics and comparison between pure intoxication and that combined with other substances of abuse. *Toxicology Letters*. 2017;277:84–91.
https://doi.org/10.1016/j.toxlet.2017.05.030

56. Sinenchenko AG, Lodyagin AN, Batotsyrenov BV, Savello VE, Antonova AM. Acute severe oral poisoning with 1,4-butandiol and ethanol with the development of a coma. *S.S. Korsakov Journal of Neurology and Psychiatry*. 2020;120(3):77–81 (In Russ.).
https://doi.org/10.17116/jnevro202012003177

57. Busardo FP, Jones AW. GHB pharmacology and toxicology: acute intoxication, concentrations in blood and urine in forensic cases and treatment of the withdrawal syndrome. *Current neuropharmacology*. 2015;13(1):47–70.
https://doi.org/10.2174/1570159X13666141210215423

58. Livanov GA, Lodyagin AN, Kaziakhmedov VA, Pochinyaeva LM, Glushkov SI, Batotsyrenov BV, et al. Clinical case of acute severe poisoning with 1,4-butanediol in a teenage girl. *Russian Journal of Anesthesiology and Reanimatology*. 2017;62(4):297–300 (In Russ.).
EDN: ZFBXJL

59. Dufayet L, Bargel S, Bonnet A, Boukerma AK, Chevallier C, Evrard M, et al. Gamma-hydroxybutyrate (GHB), 1,4-butanediol (1,4BD), and gamma-butyrolactone (GBL) intoxication: A state-of-the-art review. *Regulatory Toxicology and Pharmacology*. 2023;142:105–35.
https://doi.org/10.1016/j.yrtph.2023.105435

60. Melekhova AS, Belskaya AV, Zorina VN, Melnikova MV, Kubarskaya LG, Gaikova ON. Experimental model of convulsive syndrome based on phenylcarbamate. *Extreme Medicine*. 2024;26(4):38–48 (In Russ.).
https://doi.org/10.47183/mes.2024-26-4-38-48

61. Kurdil NV, Ivaschenko OV, Struk VF, Bogomol AG. Features of acute pesticide poisonings in urban area: carbamates, pyrethroids, neonicotinoids. *Emergency Medicine*. 2015;4(67):51–7 (In Russ.).

62. Zorina VN, Bespalov AY, Krasnov KA, Melekhova AS, Reinuk VL. Prospects for the development of new prolonged forms of valproic acid derivatives for the relief of convulsive syndrome. *S.S. Korsakov Journal of Neurology and Psychiatry*. 2022;122(7):14–9 (In Russ.).
https://doi.org/10.17116/jnevro202212207114

63. Čolović MB, Krstić DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM. Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. *Current Neuropharmacology*. 2013;13(3):315–35.
https://doi.org/10.2174/1570159x11311030006

64. Reddy BS, Skaria TG, Polepalli S, Vidyasagar S, Rao M, Kunhikatta V, et al. Factors associated with outcomes in organophosphate and carbamate poisoning: a retrospective study. *Toxicological Research*. 2020;36:257–66.
https://doi.org/10.1007/s43188-019-00029-x

65. Zorina VN, Evdokimova EA, Rejniuk VL. Methods for prevention and treatment of convulsive disorders associated with cholinergic convulsant intoxication. *Extreme Medicine*. 2022;24(2):14–21 (In Russ.).
https://doi.org/10.47183/mes.2022.019

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EFFECT OF GENERAL HYPERTHERMIA AND LOCAL COOLING ON FENTANYL TOLERANCE IN RATS

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Introduction. The toxicity of a number of xenobiotics increases with air temperature. However, it remains unknown whether this applies to narcotic analgesics and whether this dependence can be corrected by first aid measures recommended for heat stroke.
Objective. Evaluation of the effect of elevated air temperatures and local cooling on the acute toxicity of fentanyl.
Materials and methods. Three series of experiments were conducted. In the first series, the effect of elevated air temperatures on the dose dependence of the lethal and narcotic effects of fentanyl was studied. In total, 11 groups of 20 rats each were formed, which were intravenously administered fentanyl at doses of 50, 100, 200, 300, or 400 μg/kg, and one group (n = 14) without drug administration. Following fentanyl administration, one subset of rats (n = 100) was kept for 24 h at an air temperature of 22 °C; the second subset (n = 100) was kept for 40 min in a thermal chamber at 40 °C and then for 24 h at 22 °C. Those not receiving fentanyl were observed in a thermal chamber until the first case of death, then for 24 h at 22 °C. In the second series of experiments, the effect of head cooling on lethality, latent awakening time, and rectal temperature of rats (n = 49) 40 min after intravenous administration of fentanyl at a dose of 300 μg/kg (LD_5) was studied. Four groups of animals were formed, which were kept after fentanyl administration for 40 min at 22 or 40 °C with or without local cooling of the neurocranium, followed by observation for 24 h at 22 °C. In the third series of experiments, following the same scheme, the effect of cooling the middle third of the ventral surface of the torso on lethality, latent awakening time, and rectal temperature of rats (n = 48) 40 min after fentanyl administration at the same dose was studied. Statistical analysis was performed using the OriginPro software.
Results. A 40-min exposure at 40 °C was non-lethal for intact rats. After administration of fentanyl at doses of 100–400 μg/kg, lethality reached 0–5% and 60–95% at 22 °C and 40 °C, respectively. Hyperthermia induced by 40 °C exposure under fentanyl administration at a dose of 300 μg/kg was mitigated by head cooling and prevented by cooling the ventral surface of the torso. Cooling the ventral surface of the torso, rather than the head, reduced lethality from 100% to 8%. At 22 °C, both local cooling methods deepened fentanyl-induced hypothermia without significantly affecting lethality or anesthesia duration.
Conclusions. The general overheating potentiates the lethal and narcotic effects of fentanyl in rats. Under these conditions, cooling the ventral surface of the torso is an effective measure to prevent hyperthermia and lethality, while head cooling is ineffective. At room temperature, both local cooling methods deepen fentanyl-induced hypothermia without significantly affecting lethality. The efficacy of cooling the ventral surface of the torso requires evaluation not only during combined overheating but also during isolated overheating of the organism.

Keywords: lethality; local cooling; general overheating; acute intoxication; body temperature; fentanyl

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ВЛИЯНИЕ ОБЩЕГО ПЕРЕГРЕВАНИЯ И МЕСТНОГО ОХЛАЖДЕНИЯ НА ПЕРЕНОСИМОСТЬ ФЕНТАНИЛА КРЫСАМИ

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Введение. Токсичность многих ксенобиотиков возрастает с температурой воздуха, но неизвестно, относится ли это к наркотическим анальгетикам и как на эту зависимость влияют меры помощи, рекомендуемые при тепловом ударе.
Цель. Оценка влияния повышенной температуры воздуха и местного охлаждения на острую токсичность фентанила.
Материалы и методы. Проведены три серии экспериментов: в первой изучено влияние повышенной температуры воздуха на дозовую зависимость летального и наркотического действия фентанила. Формировали 11 групп по 20 крыс, которым внутривенно (в/в) вводили фентанил в дозах 50, 100, 200, 300 или 400 мкг/кг и группу (n = 14) без введения препарата. После введения фентанила часть крыс (n = 100) содержали в течение суток при температуре воздуха 22 °C; вторую часть (n = 100) — 40 мин в термокамере при 40 °C и далее в течение суток при 22 °C; не получивших фентанил наблюдали в термокамере до первого случая гибели, затем — в течение суток при 22 °C. Во второй серии изучено влияние охлаждения головы на летальность, латентное время пробуждения, ректальную температуру крыс (n = 49) через 40 мин после в/в введения фентанила в дозе 300 мкг/кг (LD_5). Формировали

4 группы животных, которых после введения фентанила содержали 40 мин при 22 или 40 °C с местным охлаждением *neurocranium* или без него и последующим наблюдением в течение суток при 22 °C. В третьей серии по такой же схеме изучено влияние охлаждения средней трети вентральной поверхности туловища на летальность, латентное время пробуждения и ректальную температуру крыс (n = 48) через 40 мин после введения фентанила в той же дозе. Статистический анализ проведен с использованием программного обеспечения OriginPro.

Результаты. Сорокаминутное пребывание интактных крыс при 40 °C было нелетальным. После введения фентанила в дозах 100–400 мкг/кг летальность составила 0–5% при 22 °C и 60–95% при 40 °C. Гипертермия при 40 °C на фоне введения фентанила в дозе 300 мкг/кг замедлялась при охлаждении головы и предотвращалась при охлаждении вентральной поверхности туловища. Охлаждение вентральной поверхности туловища, но не головы, снижало летальность со 100 до 8%. При 22 °C оба варианта местного охлаждения углубляли вызванную фентанилом гипотермию, существенно не влияя на летальность и продолжительность наркоза.

Выводы. Общее перегревание потенцирует летальное и наркотическое действие фентанила на крыс. Охлаждение в этих условиях вентральной поверхности туловища — эффективная мера предупреждения гипертермии и летальности, а охлаждение головы малоэффективно. При комнатной температуре оба варианта местного охлаждения углубляют вызванную фентанилом гипотермию, существенно не влияя на летальность. Требует оценки эффективность охлаждения вентральной поверхности туловища не только при комбинированном, но и при изолированном перегревании организма.

Ключевые слова: летальность; местное охлаждение; общее перегревание; острая интоксикация; температура тела; фентанил

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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. Проведение исследований одобрено на заседании биоэтического комитета ФГБУ НКЦТ им. С.Н. Голикова ФМБА России (протокол № 16/24 от 22.10.2024).

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INTRODUCTION

Overheating of the body is a health condition caused by an increase in body temperature under the conditions where the sum of the thermal energy released during metabolic processes and received from the environment exceeds the thermal energy lost through radiation, convection, and heat conduction.

Climatic conditions conducive to overheating of the body are not uncommon in Russia. One aspect of this problem is the associated increase in the toxicity of a number of xenobiotics for poikilothermic [1] and homeothermic animals [2]. In this regard, the influence of conditions promoting overheating of the body on the severity of side effects of narcotic analgesics is of interest. Their overdose may occur during the medical evacuation of the wounded. In addition, fentanyl derivatives are spreading rapidly on the illegal market of narcotic drugs and psychotropic substances [3]. However, there is a lack of data on predicting the effect of overheating on the toxicity of opioids. In order to fill this gap, the toxicity of such substances in cases of overdose under controlled climatic conditions should be studied.

Opioid overdose can be conveniently simulated by administering fentanyl to animals, due to a combination of short duration of action and its membership in the class of extremely toxic substances [4]. In addition, it is of interest to study the feasibility of therapeutic measures recommended for isolated overheating of the body in the form of heat stroke in the setting of opioid analgesia. The Russian Standard of Emergency Medical Care for Heat and Sun Stroke[1] prescribes the “application of an ice pack” as such a measure, although not specifying the location for its placement. In educational and methodological sources, the forehead is most often indicated as such a location[2]. The back of the head, neck, temples, collarbones, inner bends of the elbow and knee joints, calf muscles, groin, and sacral areas are also mentioned [5]; however, there is a lack of objective data characterizing the validity of such recommendations.

This study aims to evaluate the effect of elevated air temperature and local cooling on the acute toxicity of fentanyl.

MATERIALS AND METHODS

The study was conducted in November 2024 using male outbred albino rats weighing 191–210 g from the Rappolovo Laboratory Animal Breeding Facility of the Kurchatov Institute. Three series of experiments were performed.

In the first series of experiments, the effect of elevated air temperature on the dose dependence of the lethal and narcotic effects of fentanyl was studied. In total, 11 randomized groups were formed. In 10 groups, animals (n = 200) were injected with a fentanyl solution

[1] Order of the Ministry of Health of the Russian Federation No. 1115n dated 20 Dec. 2012 “Standard of Emergency Medical Care for Heat and Sun Stroke”.
[2] Bykov IYu, Rakov AE, Sosyukin AE, eds. Military Field Therapy: A National Guide. Moscow: GEOTAR-Media; 2007.

of 50 μg/mL (Moscow Endocrine Plant, batch 30212) at doses of 50, 100, 200, 300, 400 μg/kg into the lateral tail vein. In one group (*n* = 14), the drug was not administered.

The individuals that received fentanyl (*n* = 100) were left for 24 h at room temperature of 22 °C (control group); the second part of the animals (*n* = 100) was placed for 40 min in open enclosures installed in a thermal chamber at 40 °C, and then observed for 24 h at an air temperature of 22 °C.

Rats that did not receive fentanyl (dose 0 mg/kg, volume 0 mL/kg) were placed in restrainers and kept in a thermal chamber until the first case of death. After removal from the thermal chamber, observation continued for 24 h at 22 °C. During this time, awakening (at the moment of the first running movement upon tail percussion) or death was recorded. The study design is presented in the Table.

In the second series of experiments using other rats (*n* = 49), the effect of general overheating and/or local head cooling on rectal temperature, lethality, and the latent time of awakening 40 min after the administration of the officinal fentanyl preparation at a dose of 300 μg/kg was studied. This dose was preliminarily identified as LD_5 for individuals kept at room temperature. Four randomized groups were formed:

1. Group 1 (*n* = 14) — animals that received fentanyl at a dose of 300 μg/kg and were then kept for 24 h at 22 °C without local cooling;
2. Group 2 (*n* = 12) — animals that received fentanyl at a dose of 300 μg/kg, then left for 40 min at 22 °C with local cooling, and then for 24 h at 22 °C without local cooling;
3. Group 3 (*n* = 12) — animals that received fentanyl at a dose of 300 μg/kg, kept for 40 min at 40 °C without local cooling, and then for 24 h at 22 °C;
4. Group 4 (*n* = 11) — animals that received fentanyl at a dose of 300 μg/kg, kept for 40 min at 40 °C with local cooling, and then for 24 h at 22 °C.

To cool the head, plastic containers containing 70 g of melting ice were held on the surface of the *neurocranium* of the animals for 40 min after fentanyl administration, after which local cooling was stopped and body temperature was measured. The outcome of intoxication (death or awakening) was recorded over 24 h.

According to the same scheme, in the third series of experiments using four groups of 12 rats each, the influence of a 40-min stay in a thermal chamber at 40 °C and/or cooling the middle third of the ventral surface of the torso on acute fentanyl intoxication was studied. The conditions of local body cooling are illustrated in Fig. 1.

Climatic conditions promoting body overheating were simulated in a BMT Stericell SC 111 ECO thermal chamber with a capacity of 111 L (Czech Republic), which maintained an air temperature of 40 ± 1 °C, relative humidity of 48%, and an air exchange rate of 45 chamber volumes per hour. Body temperature was measured with an electric thermometer equipped with a RET-2 rat sensor (WPI, China), the tip of which was inserted into the *rectum* to a depth of 3 cm. Instrumental ophthalmoscopy was not performed; the color of the ocular fundus was indirectly assessed by the color of the eyes, which in albino rats lack pigments other than hemoglobin in the blood vessels.

Statistical analysis was performed using the OriginPro software. The results are presented as the mean value

Table. Scheme for studying the influence of conditions promoting body overheating on the dose dependence of the lethal and narcotic effects of fentanyl

Rat number	Pharmacological effects		Climate impact
	Fentanyl dose, μg/kg	Drug volume, mL/kg	
20	50	1	22 °C within 24 h after fentanyl administration
20	100	2	
20	200	4	
20	300	6	
20	400	8	
14	0	0	40 °C until the occurrence of first death, and then 22 °C for 24 h
20	50	1	40 °C for 40 min after the fentanyl administration and then for 24 h at 22 °C
20	100	2	
20	200	4	
20	300	6	
20	400	8	

Table prepared by the authors

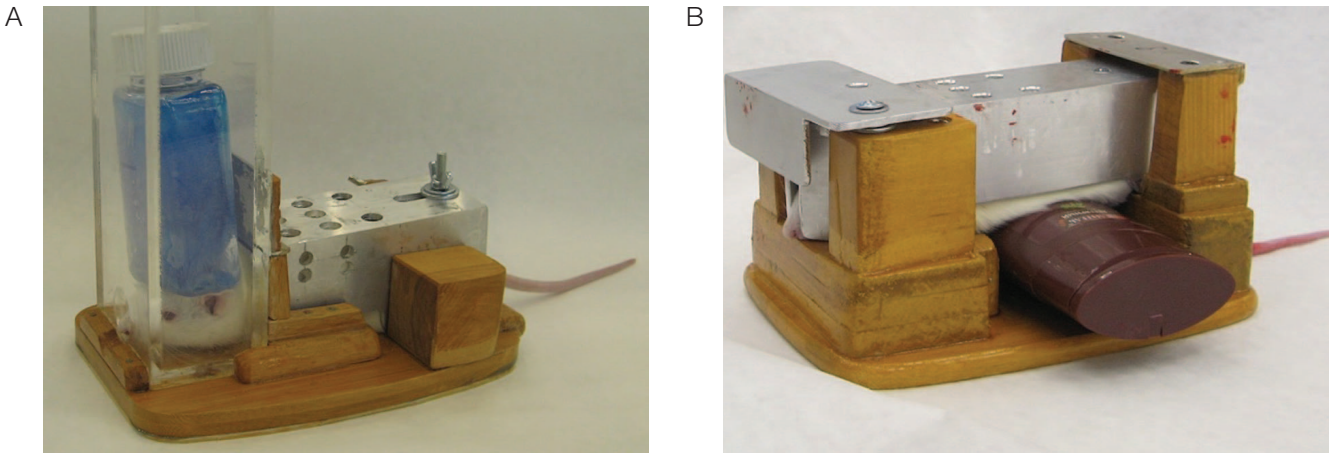

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Fig. 1. Local cooling of the rat body after fentanyl administration: A — container with melting ice is in contact with the neurocranium; B — container with melting ice is in contact with the middle third of the ventral surface of the torso

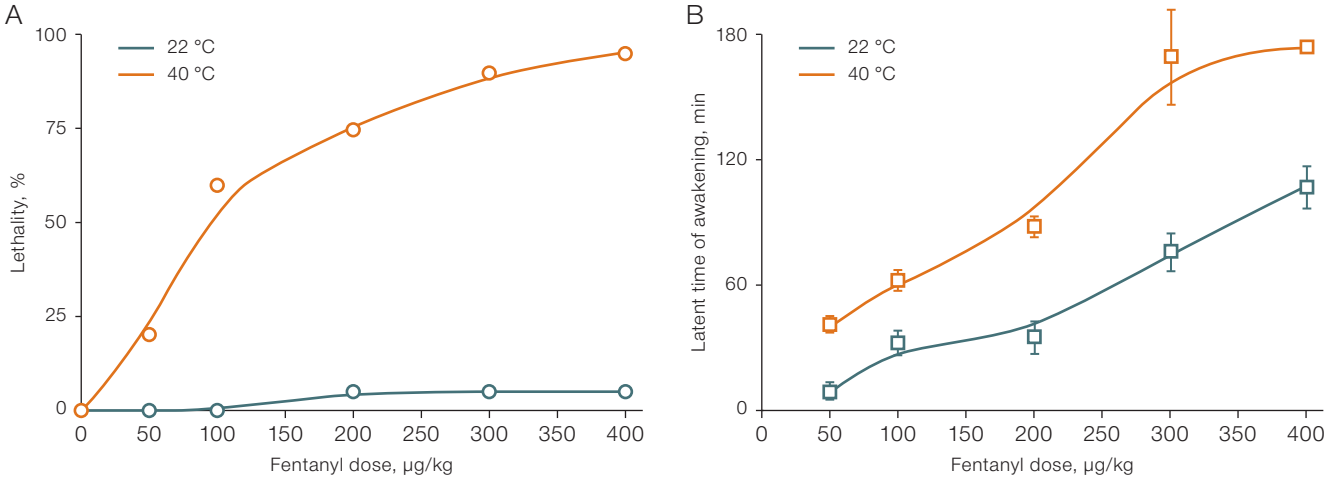

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Fig. 2. Lethality (A) and latent time of awakening (B) in rats after fentanyl administration followed by a 40-min stay at an air temperature of 22 °C or 40 °C

and its error ($M \pm m$). The Shapiro–Wilk test was used to check the normality of the distribution. The influence of the applied interventions on parametric indicators was assessed using analysis of variance. In cases of significant models, intergroup comparison of means was performed using Tukey's honestly significant difference test. LD_{50} values were calculated using the probit method. Intergroup differences in survival functions were assessed using the Gehan–Wilcoxon test, and the frequency of occurrence of alternative traits was assessed using Fisher's exact method. The critical significance level α was set at 0.05.

RESULTS

In the first series of experiments, when studying the dose dependence of the lethal and narcotic effects of fentanyl, 10–15 s after its administration, opisthotonus, tail extension, and short-term apnea followed by rare shallow breathing were observed in the rats. The ocular fundus acquired a violet color. All cases of death occurred within the first 2 h, and awakenings occurred within the first 3.4 h. For individuals kept at room temperature, fentanyl doses of 50 and 100 μg/kg were sublethal, while at doses of 200, 300, or 400 μg/kg, the lethality rate was 5%. Thus, at room air temperature, the LD_{50} value for fentanyl administered intravenously as an official solution in a volume acceptable for rats (≤ 2 mL) was not reached.

In animals placed in the thermal chamber for 40 min after fentanyl administration, the LD_{50} was 113 ± 16 μg/kg (Fig. 2A). Thermal exposure against the background of fentanyl administration increased the latent time to awakening by 1.6–4.0 times (from 10–110 to 40–176 min, Fig. 2B). In such animals, transient ataxia was observed after awakening, which was absent in the control group.

For rats that did not receive fentanyl, the 40-min thermal exposure was non-lethal; the first case of death was noted only 90 min after being placed in the thermal chamber.

Figure 2 shows that, compared to animals left at room temperature, the difference is significant in the dose range of 100–400 μg/kg for lethality and in the dose range of 50–400 μg/kg for the latent time of awakening, $p < 0.05$.

In the second and third series of experiments, when studying the thermal state of the body, the initial rectal temperature was 38.1 ± 0.1 °C (n = 96). Thus, 40 min after fentanyl administration, the rectal temperature of surviving individuals in Group 1, left at room temperature without local body cooling, decreased to 34.5 ± 0.2 °C (n = 25), while in rats from Group 3 (n = 5) that remained alive at this time, it increased to 44.5 ± 0.4 °C. With head cooling, the body temperature in rats left at room temperature was 33.7 ± 0.3 °C, Group 2 (n = 10), and in the six surviving individuals from Group 4 at the time of removal from the thermal chamber, it was 39.8 ± 0.6 °C (Fig. 3A). Cooling the ventral surface of the torso reduced the body temperature of animals compared to those not receiving local cooling in animals that, after fentanyl administration, were kept at both room and elevated air temperatures: to 29.7 ± 0.3 °C (n = 9) and 33.0 ± 0.5 °C (n = 11) over 40 min, respectively. Over 40 min, the difference in mean group body temperature values among the rats that survived exposure to the thermal chamber, without and with local cooling of the ventral torso surface, reached 11.4 °C (Fig. 3B).

In rats left at room temperature after fentanyl administration, lethality was 7–8%, while in those placed in a thermal chamber for 40 min, it was 100% ($p < 0.05$). The effect of head cooling on lethality showed a tendency to increase by 10% in rats left at room temperature and to decrease by 27% in those placed in the thermal chamber (Fig. 4A).

Cooling the ventral surface of the torso completely prevented the aggravating effect of elevated air temperature on acute intoxication. Thus, the lethality decreased by 92% and was similar to that in rats left at room temperature without local cooling (Fig. 4B). In animals left at room temperature after fentanyl administration, cooling the ventral surface of the torso showed a tendency to increase lethality by 17% compared to those without local cooling. Rats subjected to thermal exposure after fentanyl administration without local body cooling died without awakening. No significant intergroup differences in the latent time of awakening were recorded among the remaining animals, who did not show signs of ataxia.

DISCUSSION

The range of fentanyl doses used in the present study, 50–400 μg/kg, included the ED_{100} for analgesic activity in rats: 75 μg/kg [6]. When calculated based on body surface area, the administered doses are bioequivalent to 8–67 μg/kg for humans. This is 1–8 times higher than the doses used for neuroleptanalgesia and 2–16 times higher than the doses used in surgeries with spontaneous breathing[3], modelling an overdose of an opioid narcotic analgesic.

While unlikely in a hospital setting, such an overdose is possible due to lapses in the continuity of care for the wounded during stages of medical evacuation, including under conditions promoting body overheating. In humans performing light physical work, overheating occurs when

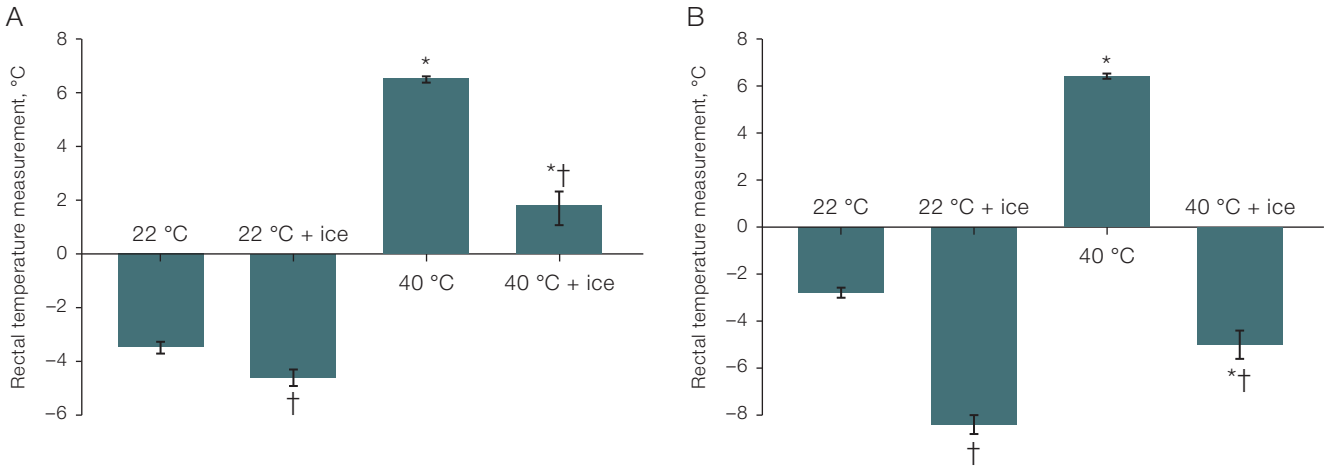

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Fig. 3. Changes in the rectal temperature of rats after fentanyl administration at a dose of 300 μg/kg and a 40-min stay at an air temperature of 22 °C or 40 °C with ice cooling of the head (A) or the ventral surface of the torso (B)

Note: statistically significant difference ($p < 0.05$): * — compared to the corresponding group of rats left at room temperature after fentanyl administration; † — compared to the corresponding group of rats without local cooling.

[3] Mashkovsky MD. Medicinal Products. Moscow: Novaya Volna; 2021.

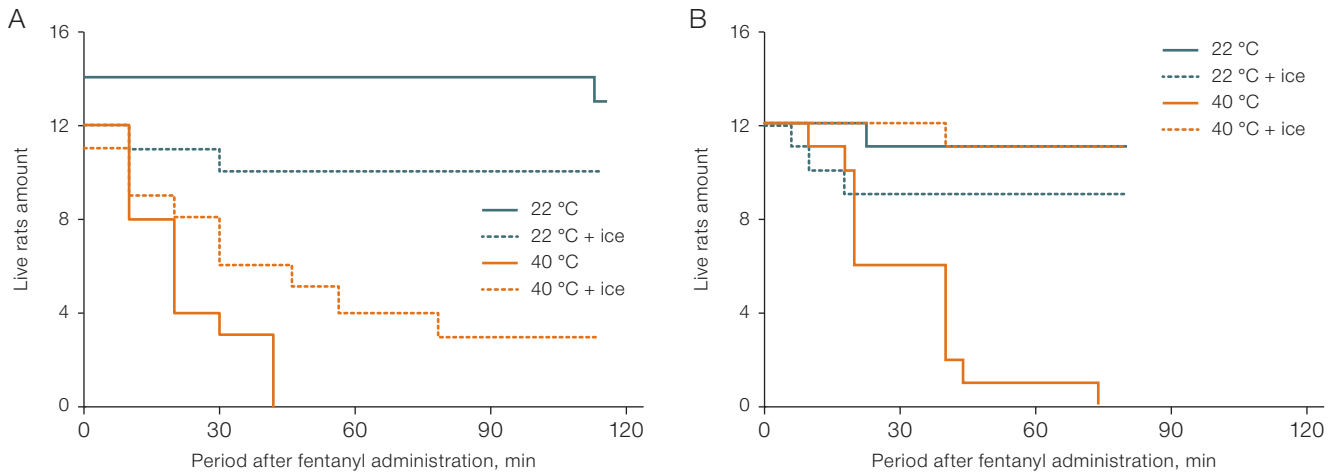

Figure prepared by the authors

Fig. 4. Survival of rats after fentanyl administration at a dose of 300 μg/kg and a 40-min stay at an air temperature of 22 °C or 40 °C with ice cooling of the head (A) or the ventral surface of the torso (B)

Note: statistically significant effect ($p < 0.05$) was found for: general overheating without local cooling (A, B); local cooling during general overheating (B).

the air temperature exceeds 31 °C as measured by a "wet" bulb thermometer [7], which, at a relative humidity of 48%, corresponds to 40 °C on a "dry" bulb thermometer. According to the data obtained, a 40-min exposure to such conditions was easily tolerated by intact rats but severely exacerbated the toxic effects of opioids at doses that are non-lethal under thermal comfort conditions. These data characterize the type of interaction between the undesirable side effects of the opioid and conditions promoting body overheating as potentiation.

Overheating in animals placed in a thermal chamber after fentanyl administration occurred despite its hypothermic effect observed at room temperature [8]. In the setting of fentanyl exposure, overheating may have been facilitated by impaired evaporative heat loss from the body surface, which is hypothetically achieved in rats by spreading saliva onto their fur [9]. Narcotization deprived the animals of this thermoregulatory mechanism, analogous to sweat evaporation in humans. Reduced evaporation of moisture from the respiratory tract surface due to suppressed external respiration may also have contributed to decreased heat loss.

Exposure to a thermal chamber increased the rectal temperature of anesthetized rats to 44.5 °C, exceeding the threshold for irreversible tissue damage (43 °C) by 1.5 °C [10]. This was a sufficient condition for their death. In comparison, without thermal exposure, the administration of an equivalent dose of fentanyl was non-lethal for most animals. Reversible impairments, such as increased blood–brain barrier permeability and elevated brain water content, can occur even at lower body temperatures (42 °C) [10]; these may have been associated with the ataxia observed in rats that awakened after fentanyl administration and thermal chamber exposure. Thus, in the absence of local body cooling, hyperpyrexia was the factor precluding survival at an air temperature of 40 °C during acute fentanyl intoxication.

When the ventral torso surface or head was cooled, the body temperature did not reach 42 °C during the stay of animals in a thermal chamber. Although head cooling prevented hyperpyrexia during thermal chamber exposure, it did not significantly affect lethality. Probable mechanisms of thanatogenesis in these rats included cerebral edema and swelling, which have been described in both severe opioid intoxications [11] and heat stroke [12]. An increase in brain volume within the confined space of the skull leads to elevated intracranial pressure and reduced cerebral blood flow velocity. This may explain the lower cooling efficacy of ice application to the head compared to the ventral torso surface. These findings appear to contradict reports of high cerebral blood flow velocity[4], high heat flux density from the head surface [13], and recommendations to cool the head during body overheating [5, 14]. This contradiction may be explained by the hypothesis of reduced cerebral blood flow velocity, which impeded heat transfer from the body thermal "core" to the cooled surface of the neurocranium during acute fentanyl intoxication under conditions of body overheating.

Cooling the middle third of the ventral surface of the torso proved to be a highly effective measure for preventing body overheating despite elevated air temperatures and reduced evaporative heat loss. This indicates the sufficiency of the volumetric blood flow rate in the visceral region for convective transfer to the cooled surface not only of excess heat entering the body from the external environment but also of heat released during metabolic processes. In light of this, it seems expedient to evaluate the efficacy of local abdominal cooling not only against the background of opioid exposure but

[4] Osipov AP, Ibishov DF, Rastorguyeva SL. Physiology of Blood Circulation and Lymph Circulation. Textbook. Perm: IPC "Prokrost"; 2022.

also during isolated body overheating, with potential revisions to the current "Standard of Emergency Medical Care for Heat and Sun Stroke" if necessary.

However, the data obtained also indicate that local body cooling should not be recommended during acute opioid intoxication under comfortable climatic conditions. It is likely that the decrease in body temperature during local cooling, combined with fentanyl-induced peripheral vasodilation [15] and its direct inhibitory effect on mitochondrial oxygen consumption [16], suppressed the respiratory center and exacerbated hypoxemia, as indicated by cyanosis of the ocular fundus of the animals.

The findings allow us to formulate a hypothesis regarding the mechanisms by which body overheating and cooling of the ventral torso surface against this background affect the toxic effects of fentanyl. Both opioids and heat stress inhibit the propulsive function of the gastrointestinal tract, promoting the overgrowth of thermophilic microflora and its production of toxic substances, including ammonia and lower amines [17]. The accumulation of fluid in the intestinal lumen associated with activation of μ-opioid receptors by fentanyl also stimulates bacterial growth, further facilitating the formation of these substances [18]. The translocation of ammonia and lower amines from the gastrointestinal chyme into the bloodstream is enhanced by μ-opioid receptor agonist-mediated increased intestinal epithelial permeability, which is mediated by Toll-like receptor activation [18]. The subsequent transfer of ammonia and lower amines from the blood into the brain may be facilitated by metabolic acidosis caused by body overheating and acute respiratory failure. The characteristic increase in the pH gradient between blood plasma and cytoplasm intensifies the entry of ammonia and lower amines from the blood into astroglia along the concentration gradient of their neutral gaseous forms [19], which should lead to the accumulation of free amino acids in the brain [20] and cerebral swelling. Due to the inverse relationship between water temperature and the solubility of gases (including ammonia and lower amines), such swelling cannot be alleviated by local head cooling. Conversely, cooling the gastric and intestinal chyme by the magnitude observed in this study — an 11.4 °C drop in body temperature during ice application to the ventral torso surface – should reduce the metabolic activity of thermophilic gastrointestinal microflora by at least 2.2 times, thereby blocking the mechanisms of thanatogenesis mediated by acute intestinal endotoxemia. Testing this hypothesis in further research opens prospects for new approaches to the pathogenetic therapy of both acute opioid intoxication and heat stroke.

CONCLUSIONS

1. Body overheating potentiates the lethal and narcotic effects of fentanyl in rats. Under these conditions, cooling the ventral surface of the torso is an effective measure to prevent hyperthermia and reduce lethality, while head cooling is ineffective.

2. At room air temperature, local cooling of both the head and the ventral surface of the torso exacerbates fentanyl-induced hypothermia without significantly affecting rat lethality.

3. The data obtained should be considered in further research into the influence of climatic factors on the tolerability of narcotic analgesics in larger laboratory animals and humans.

References

1. Acs A, Schmidt J, Németh Z, Fodor I, Farkas A. Elevated temperature increases the susceptibility of *D. magna* to environmental mixtures of carbamazepine, tramadol and citalopram. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*. 2025;287:110052. https://doi.org/10.1016/j.cbpc.2024.110052
2. Gordon CJ, Johnstone AFM, Aydin C. Thermal stress and toxicity. *Comprehensive Physiology*. 2014;4(3):995–1016. https://doi.org/10.1002/cphy.c130046
3. Golovko AI, Ivnitsky JuJu, Rejniuk VL, Schäfer TV. Changes in the illegal synthetic opioids market. *Narcology*. 2024;23(2):54–9 (In Russ.). EDN: SEMZIT
4. Berezovskaya IV. Prediction of drug safety in preclinical toxicological studies. *Toxicological Bulletin*. 2010;(5):17–22 (In Russ.). EDN: TQUNAB
5. Shabalov NP, ed. *Pediatrics*. SPb.: SpecLit; 2003 (In Russ.). EDN: QLFJWL
6. Getsy PM, May WJ, Young AP, Baby SM, Coffee GA, Bates JN, et al. Tropine exacerbates the ventilator depressant actions of fentanyl in freely-moving rats. *Frontiers in Pharmacology*. 2024;24:1405461. https://doi.org/10.3389/fphar.2024.1405461
7. Wolf ST, Bernard TE, Kenney WL. Heat exposure limits for young unacclimatized males and females at low and high humidity. *Journal of Occupational and Environmental Hygiene*. 2022;19(7):415–24. https://doi.org/10.1080/15459624.2022.2076859
8. Canfield JR, Sprague JE. Influence of carbon side chain length on the in vivo pharmacokinetic and pharmacodynamics characteristics of illicitly manufactured fentanyls. *Drug Testing and Analyses*. 2024;16(10):1113–21. https://doi.org/10.1002/dta.3636
9. Attah AT, Negrón-Moreno PN, Amigo-Duran M, Zhang L, Kenngott M, Brecht M, et al. Sensory cues, behavior and fur-based drying in the rat wetness response. *Scientific Reports*. 2024;14(1):24550. https://doi.org/10.1038/s41598-024-74900-9
10. Yarmolenko PS, Moon EJ, Landon C, Manzoor A, Hochman DW, Viglianti L, et al. Thresholds for thermal damage to normal tissues: an update. *International Journal of Hyperthermia*. 2011;27(4):320–43. https://doi.org/10.3109/02656736.2010.534527
11. Puszkei A, Malissin I, Cisternino S, Pallet N, Declèves X, Mégarbane B. Massive tramadol ingestion resulting in fatal injury — a pharmacokinetic study with discussion on

the involved mechanisms of toxicity. *Clinical Toxicology*. 2022;60(9):1059–62. https://doi.org/10.1080/15563650.2022.2071286
12. Lee S, Lee SH. Exertional heat stroke with reversible severe cerebral edema. *Clinical and Experimental Emergency Medicine*. 2021;8(3):242–5. https://doi.org/10.15441/ceem.19.085
13. Alali Z, Eckels SJ. 3D numerical simulations of mixed convective heat transfer and correlation development for a thermal manikin head. *Heliyon*. 2024;10(9):e30161.
14. Wang J, Jiang C, Kang J, Yu S, Bai G. Head-neck local ventilation mode for long-narrow mine working face. *Scientific Reports*. 2024;14(1):19663. https://doi.org/10.1038/s41598-024-70739-2
15. Solis E, Cameron-Burr KT, Shaham Y, Kiyatkin EA. Fentanyl-induced brain hypoxia triggers brain hyperglycemia and biphasic changes in brain temperature. *Neuropsychopharmacology*. 2018;43(4):810–9. https://doi.org/10.1038/npp.2017.181
16. Zamparelli M, Eaton S, Quant PA, McEwan A, Spitz L, Pierro A. Analgesic doses of fentanyl impair oxidative metabolism of neonatal hepatocytes. *Journal of Pediatric Surgery*. 1999;34(2):260–3. https://doi.org/10.1016/s0022-3468(99)90186-0
17. Ivnitsky JuJu, Rejniuk VL, Schäfer TV, Vakunenkova OA. Acute intestinal endotoxicosis in disaster medicine. *Kremlin Medicine Journal.* 2024;1:81–6 (In Russ.). https://doi.org/10.48612/cgma/1e9k-uttr-7h6a
18. Lacy BE, Cangemi DJ. Opioids and the gastrointestinal tract: the role of peripherally active μ-opioid receptor antagonists in modulating intestinal permeability. *American Journal of Gastroenterology*. 2024;119(10):1970–8. https://doi.org/10.14309/ajg.0000000000002887
19. Ott P, Vilstrup H. Cerebral effects of ammonia in liver disease: current hypotheses. *Metabolic Brain Disease*. 2014;29(4):901–11. https://doi.org/10.1007/s11011-014-9494-7
20. Zielinska M, Albrecht J, Popek M. Disregulation of astrocytic glutamine transport in acute hyperammonemic brain edema. *Frontiers in Neuroscience.* 2022;16:875750. https://doi.org/10.3389/fnins.2022.874750

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ON THE ISSUE OF LEGAL REGULATION AND METHODOLOGICAL SUPPORT FOR THE ACTIVITIES OF JOINT MEDICAL TEAMS OF FMBA OF RUSSIA

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Introduction. Modern challenges to the national security of Russia necessitate improvements in the medical and sanitary support system, including the medical personnel and resources of the Federal Medical and Biological Agency (FMBA) of Russia during emergency situations, terrorist attacks, and armed conflicts. Special emphasis is placed on enhancing the promptness, effectiveness, and organization of providing specialized medical assistance and carrying out medical evacuations for victims, injured persons, and patients. This need is underscored by the existing experience of Joint Medical Teams (JMTs) of the FMBA of Russia participating in the elimination of large-scale emergency consequences and ongoing Special Military Operation (SMO).
Objective. Development of proposals for improving the regulatory framework and methodological support for the creation, preparation, use, and operation of FMBA JMTs.
Materials and methods. The study utilized normative and methodological documents of the Agency, reports from district medical centers (DMCs), and data from a survey questionnaire involving 192 medical specialists engaged in the activities of JMTs over the period from February 2022 to June 2025, including their involvement in the SMO. The following methods were employed in conducting this research: formal legal analysis, content analysis, expert evaluation, statistical processing of data, as well as logical and information modeling techniques. The study encompassed legal, organizational, and methodological aspects related to the functioning of JMTs in various types of emergency situations. The analysis took into account extensive experience in mitigating health-related consequences of emergencies and the role of such teams in facilitating medical evacuation, delivering medical aid to affected individuals, wounded soldiers, and ill servicemen.
Results. Gaps in the normative and methodological support for the activities of FMBA JMTs were established, despite their relatively high response efficiency and functional effectiveness, confirmed by their participation in providing medical support during the SMO. The expert assessments among 192 medical professionals, most of whom (60.4%) had practical experience of working within JMTs, showed dissatisfaction with the current normative and methodological basis. According to the experts, the current framework does not provide for sufficient clarity or regulation for establishing, preparing, utilizing, maintaining, and implementing medical evacuation processes by JMTs. The collected data indicate a pressing need for a comprehensive improvement of the legal and methodological basis for JMT activities. These improvements include drafting and adopting a single Standardized Regulation for JMTs, supplementing FMBA Order No. 126 dated 25.04.2022, taking into account specific tasks related to medical evacuation, entrusting DMCs with responsibilities for forming, training, and managing JMTs, and elaborating detailed methodological recommendations aimed at standardizing the organizational structure, equipping standards, and preparedness criteria for JMTs. The results obtained form a basis for resolving numerous problematic issues impeding effective functioning of JMTs.
Conclusions. The results obtained indicate gaps in the normative and methodological framework regulating the activities of FMBA JMTs, which hinder the development of clear mechanisms for their formation, application, and functioning when organizing and providing medical assistance to victims of emergencies, terrorist acts, and military conflicts. Implementation of the proposed measures will enhance the efficiency and effectiveness of JMTs within the healthcare system, contributing significantly to national security interests.

Keywords: joint medical team; FMBA of Russia; normative and methodological support; emergency situation; special military operation; medical evacuation; regional medical center; national security

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К ВОПРОСУ О НОРМАТИВНОМ РЕГУЛИРОВАНИИ И МЕТОДИЧЕСКОМ СОПРОВОЖДЕНИИ ДЕЯТЕЛЬНОСТИ СВОДНОГО МЕДИЦИНСКОГО ОТРЯДА ФМБА РОССИИ

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Введение. Современные вызовы национальной безопасности требуют совершенствования системы медико-санитарного обеспечения, в том числе медицинских сил и средств Федерального медико-биологического агентства (далее — ФМБА России, Агентство) в условиях чрезвычайных ситуаций (ЧС), террористических актов и вооруженных конфликтов. Особую значимость приобретает

повышение оперативности, эффективности и организованности оказания специализированной медицинской помощи и проведения медицинской эвакуации пострадавших, раненых и больных, что подтверждается имеющимся опытом участия сводных медицинских отрядов (СМО) ФМБА России в ликвидации последствий крупных ЧС и проводимой специальной военной операции (СВО).
Цель. Разработка предложений по совершенствованию нормативно-правовой базы и методического обеспечения создания, подготовки, применения и функционирования СМО ФМБА России.
Материалы и методы. В исследовании использованы нормативные и методические документы Агентства, отчетные материалы окружных медицинских центров (ОМЦ) и данные анкетного опроса 192 медицинских специалистов, вовлеченных в деятельность сводных медицинских отрядов в период 02.2022–06.2025, включая опыт участия в специальной военной операции. При выполнении исследования применялись следующие методы: формально-юридический и контент-анализ, экспертная оценка, статистическая обработка данных, а также методы логического и информационного моделирования. Исследование охватывало правовые, организационные и методические аспекты функционирования СМО в условиях чрезвычайных ситуаций различного характера. Анализ проводился с учетом обширного опыта ликвидации медико-санитарных последствий ЧС и участия СМО в осуществлении медицинской эвакуации, оказании медицинской помощи пострадавшим, раненым и больным военнослужащим.
Результаты. Выявлены пробелы в нормативно-методическом обеспечении деятельности СМО ФМБА России, несмотря на их достаточно высокую оперативность реагирования и эффективность функционирования по предназначению, подтвержденные опытом участия в медицинском обеспечении специальной военной операции. Экспертная оценка 192 медицинских специалистов, большая часть которых (60,4%) имела реальный практический опыт работы в составе СМО, показала низкий уровень удовлетворенности действующей нормативно-методической базой, не обеспечивающей в полной мере четкого регулирования порядка создания, подготовки, применения, функционирования и выполнения медицинской эвакуации СМО. На основании полученных данных обоснована необходимость комплексного совершенствования правовых и методических основ деятельности СМО, заключающаяся в разработке и утверждении единого Типового положения о сводном медицинском отряде, внесении дополнений в приказ ФМБА России от 25.04.2022 № 126 с учетом задач по медицинской эвакуации, закреплению за ОМЦ функции по формированию, подготовке и управлению СМО, а также разработке детализированных методических рекомендаций, стандартизирующих организационную структуру, оснащение и критерии готовности СМО. Реализация результатов исследования обеспечит создание условий для разрешения многих проблемных вопросов, имеющих высокую практическую значимость для СМО.
Выводы. Полученные результаты исследования свидетельствуют о наличии недочетов в нормативно-методической базе, определяющей порядок деятельности СМО, которые препятствуют созданию четких механизмов регулирования формирования, применения и функционирования СМО при организации и оказания медицинской помощи пострадавшим в результате чрезвычайных ситуаций, террористических актов и военных конфликтов. Реализация предложенных мер позволит повысить оперативность и эффективность применения СМО в системе медико-санитарного обеспечения в интересах национальной безопасности.

Ключевые слова: сводный медицинский отряд; ФМБА России; нормативно-методическое обеспечение; чрезвычайная ситуация; специальная военная операция; медицинская эвакуация; окружной медицинский центр; национальная безопасность

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Финансирование: работа выполнена без спонсорской поддержки.

Соответствие принципам этики: авторы заявляют, что одобрение комитетом по этике не требовалось, поскольку были проанализированы обезличенные данные анкет социологического обследования проблемных организационных вопросов здравоохранения.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The implementation of the National Security Strategy of the Russian Federation involves the development, adjustment, and implementation of a set of diverse state programs aimed at ensuring safety and sustainable development of the country.[1] This applies in full measure to the development and improvement of medical personnel and resources of the Federal Medical and Biological Agency (FMBA)[2] of Russia, enhancing the quality of medical and sanitary services provided to workers in industries with particularly hazardous working conditions, as well as residents living in service areas. Special attention is given to preserving and strengthening public health, extending professional activity and overall life expectancy. In this context, enhancing the level of timely response, as well as the effectiveness and efficiency of preventive measures, localization, and mitigation of medical and sanitary consequences resulting from incidents, accidents, disasters, natural calamities, and other emergencies of different nature becomes particularly important[3].

Current challenges demand continuous enhancement of the capabilities of medical units of the FMBA in the context of ensuring national security [1–4]. Key priorities remain improving the quality of medical care provided and ensuring readiness for efficient responses to terrorist attacks, armed conflicts, and major

[1] Presidential Decree No. 400 “On the Strategy of National Security of the Russian Federation” dated 02.07.2021.
[2] Decree of the President of the Russian Federation No. 568 “Issues Concerning the Federal Medical and Biological Agency” dated 02.07.2024
[3] Order of FMBA of Russia No. 144 “On Approval of the Regulation on Functional Subsystem of Medical-Sanitary Assistance to Victims in Emergencies in Organizations (Facilities) Under the Jurisdiction of FMBA of Russia, as Well as in Organizations and Territories Serviced by FMBA of Russia” dated 20.05.2022.

emergencies.[4,5,6,7] The likelihood of terrorist attacks and armed conflicts accompanied by a significant number of casualties remains high. Victims belong to various population categories and require timely specialized medical care and organized medical evacuation, which should be carried out to specialized stationary treatment facilities using different modes of transport [5–10].

Mobile medical formations, specifically joint medical teams (JMTs), play a crucial role in addressing the medical and sanitary consequences of large-scale emergencies and armed conflicts.[8] Such units primarily provide urgent medical care and conduct medical evacuation of victims, wounded, and patients while adhering to routing principles [5–11]. Practice has confirmed the effectiveness of JMTs but also highlighted the need for their improved normative and methodological support. Optimizing the management system of subordinate organizations of the FMBA of Russia and establishing effective interaction with government authorities requires refinement of the legal framework regulating the activities of JMTs. Therefore, studying the current state of normative regulation and methodological support for their activities becomes particularly relevant. Additionally, an analysis of existing approaches can contribute to the development and modernization of the medical assistance management system in emergency situations as a whole [12–16].

In this study, our aim was to develop proposals for improving the legal framework and methodological support for the creation, preparation, deployment, and operation of FMBA JMTs.

MATERIALS AND METHODS

We used normative and methodological documents of the FMBA regulating the activities of JMTs, including FMBA orders No. 60 dated 28.02.2022, No. 35 dated 29.02.2024, and No. 1263 dated 25.04.2022, as well as the draft Regulations on the Organization and Coordination of Activities of JMTs of FMBA of Russia (2023), developed by the Center for Strategic Planning and Management of Medical and Biological Health Risks. Additionally, reports from district medical centers (DMCs) of FMBA were analyzed, covering the formation, deployment, and operation of JMTs (February 2022–April 2025) during the Special Military Operation (SMO). The study also included data from surveys in the form of questionnaires assessing the condition of formation and functioning of JMTs. The questionnaires were filled out by medical experts directly involved in the work of these teams, including those who participated in responding to medical and sanitary consequences of emergencies, terrorist attacks, and military conflicts.

The formulated objectives were achieved by a set of methods, including a formal and legal analysis of normative documents, a content analysis of texts identifying gaps and contradictions, expert evaluations involving 192 medical professionals. The latter were divided into two groups: Group 1 (n = 116) comprised individuals with practical experience working in JMTs, while Group 2 (n = 76) comprised specialists involved in forming the teams but not participating in actual emergency situations. Statistical data were processed using the SPSS Statistics 23.0 software package and Microsoft Excel 2010. Statistical data processing allowed identification of patterns in assessments of the normative regulation and methodological support for JMTs. When developing proposals for improvement, methods of logical and informational modeling, as well as analytical methods ensuring systematic generalization of the obtained results, were employed. The study covers both legal aspects of regulating the activities of JMTs, as well as organizational and methodological issues related to the preparation, functioning, and management of JMTs in various types of emergency situations.

RESULTS

At the initial stage of the study, we assessed the level of satisfaction of medical professionals regarding the current state of normative regulations and methodological support for the activity of JMTs. The results of expert evaluations indicate that, according to contemporary requirements for safety and operational conditions of JMTs, the current normative and methodological framework regulating the order of creation, preparation, deployment, and functioning of JMTs is less than optimal (Table).

Only 24.0% of the experts assessed the current normative and methodological framework as fully supporting the activities JMTs. More than 66.6% of the respondents (31.2% from Group 1 and 35.4% from Group 2) indicated limited or partial compliance of documents with the current requirements. Notably, 79.3% experts from Group 1, who have had the practical experience of working in JMTs, believe that existing norms and methodologies do not adequately meet the needs of such teams. At the same time, 47.3% of experts from Group 2 evaluated the normative and methodological support positively, confirming the influence of practical experience on objectivity.

[4] Order of FMBA of Russia No. 144 “On Approval of the Regulation on Functional Subsystem of Medical-Sanitary Assistance to Victims in Emergencies in Organizations (Facilities) Under the Jurisdiction of FMBA of Russia, as Well as in Organizations and Territories Serviced by FMBA of Russia” dated 20.05.2022.

[5] Order of FMBA of Russia No. 60 “On Formation of Joint Medical Teams of FMBA of Russia and Improving Readiness of Medical Institutions of FMBA of Russia for Work in Emergency Situations” dated 28.02.2022.

[6] Order of FMBA of Russia No. 35 “6. Amendment to the Order of FMBA of Russia No. 60 dated 28.02.2022 ‘On Forming Joint Medical Teams of FMBA of Russia for Operations in Emergency Situations’” dated 29.02.2024.

[7] Regulation on Organization and Coordination of Joint Medical Teams of the Federal Medical and Biological Agency (Draft). Moscow, 2023.

[8] Order of FMBA of Russia No. 126 dated 25.04.2022 “On Approval of Cases and Procedure for Organizing Provision of Medical and Sanitary Care and Specialized, Including High-Tech, Medical Aid by Medical Staff of Medical Institutions Subordinate to FMBA of Russia Beyond Such Medical Institutions”.

Table. Distribution of expert opinions regarding the state of normative regulation and methodological support for the activity of FMBA JMTs

Assessment of normative and methodological support	Share of experts who selected the variant from the total number of experts, % (*n* = 192)	Including the assessment of this particular group of experts, %	
		first (*n* = 116)	second (*n* = 76)
Provide the entire scope of activities	24.0	13.8	39.5
Provide on a limited basis	31.2	34.5	26.3
Provide partially	35.4	44.8	21.0
Difficult to answer	9.4	6.9	13.2
Total:	100.0	100.0	100.0

Table compiled by authors based on their own data

Note: *n* — number of experts.

Difficulties in assessment noted by 6.9–13.2% of the respondents can be explained by insufficient practical experience before the SMO. Then, JMTs were occasionally deployed for small-scale emergency response efforts, preventing most specialists from gaining adequate and objective experience in such conditions.

Completely different conditions emerged during the SMO course for testing the quality of normative and methodological documents. Large-scale, prolonged, and multifaceted employment of JMTs in combat zones enabled a substantial number of medical personnel to gain substantive experience, including practical verification of provisions outlined in regulatory documents. It was precisely this hands-on experience that became a reliable criterion for evaluating the comprehensiveness, clarity, and applicability of these documents.

The experience gained by JMTs during the SMO revealed gaps in the existing documentation. Only 15 (12.8%) experts involved in forming squadrons for the SMO reported complete and clear normative provisions regarding structure, goals, establishment process, and functionality. Meanwhile, 59 (51.3%) experts pointed to a partial coverage, 24 (20.5%) mentioned the lack of essential documents, and 18 (15.4%) found it difficult to respond. A significant proportion of the experts expressed the need for comprehensive regulation: 164 (85.4%) supported the idea of developing and validating an all-inclusive policy document. On the contrary, only 13 (6.8%) saw no need for such changes, while 15 (7.8%) remained undecided. Similarly, 143 (74.5%) experts backed amendments to FMBA Order No. 126[9] dated 25.04.2022, concerning the creation, usage, and function of JMTs in handling the aftermath of emergencies, terrorist attacks, and armed conflicts, including integration into general therapeutic-evacuation systems for injured and ill military personnel. Among those who opposed and abstained were 29 (15.1%) and 20 (10.4%) experts, respectively.

DISCUSSION

The data obtained allow us to conclude that FMBA JMTs are becoming increasingly important in the system of medical and sanitary provision for dealing with the consequences of emergencies, terrorist acts, and armed conflicts.

According to the information presented by V.I. Skvortsova, Head of the FMBA of Russia, throughout its participation in the SMO (02.2022–04.2025), medical teams assisted 143,000 injured military personnel, with 12,000 undergoing surgeries.[10] These figures highlight not only the scale of engagement of FMBA medical units but also indicate a qualitative change in the type of medical care provided. As emphasized by V.I. Skvortsova, “the structure of medical assistance has changed, with surgical interventions now being the primary focus.”[11]

In 2024, the volume of medical care provided by FMBA medical professionals increased by 65%, and the number of surgeries more than tripled compared to previous years.[12] This trend reflects both the rising load on the FMBA medical staff and resources and the enhanced operational readiness and functional

[9] Order of FMBA of Russia No. 126 “On Approval of Cases and Procedures for Providing Medical and Sanitary Care, Specialized, Including High-Tech Medical Care, by Healthcare Professionals of Medical Facilities Subordinated to FMBA of Russia Outside These Facilities” dated 25.04.2022.

[10] FMBA medical teams provided medical assistance to 143,000 servicemen in the SMO zone. Kommersant; 2024. URL: https://www.kommersant.ru/doc/7693365 (request date: 08.08.2025).

[11] Ibid.

[12] Ibid.

sustainability of these teams. Notably, medical "special forces" of FMBA, according to V.I. Skvortsova, participated in nearly all major emergency incidents in Russia in 2024, highlighting the expansion of JMT functions and their integration into the national emergency response system.[13]

Extensive and sustained deployment of JMTs during the SMO uncovered deficiencies in their normative and methodological support. Most experts with practical experience in JMTs rated the normative framework as requiring further development and improvement. Serious concerns arise due to the absence of clear provisions in the current regulations defining the procedure for creating, preparing, functioning, equipping, and managing JMTs.

FMBA DMCs have demonstrated particular importance. It is on their basis that JMTs are formed. However, Order No. 208 of the FMBA of Russia dated 30.07.2020, which defines their functions, does not officially assign DMCs a role in the formation, training, and management of JMTs.[14] This creates organizational and legal uncertainties and reduces accountability for the readiness of such teams. It is noteworthy that 80.7% of the experts supported assigning DMCs functions such as organizing the creation and training of JMTs, providing methodological support for their activities, monitoring readiness, and ensuring their deployment in emergency situations.

Another significant gap is the legal regulation of medical evacuation. Agency Order No. 126 dated 25.04.2022[15] provides for the formation of mobile medical teams to deliver medical care outside assigned territories; however, it does not regulate the procedure for medical evacuation. According to S.V. Markov, by early 2025, specialists from the Burnazian Federal Medical Biophysical Center alone had evacuated over 32,000 patients, including more than 7000 by air transport [6].

On the basis of the data obtained from the application of JMTs in the SMO and emergency situations, several practical steps may be recommended:

- establishment of typical organizational structures for JMTs tailored to the profile of emergency (chemical, radiation, biological, terrorism, warfare);
- determination of composition and equipment standards for medical brigades;
- development of protocols for functioning, including organization of medical care and evacuation along routing principles;
- codification of technologies for training and professional coordination;
- clarification of regulations for creation, replenishment, and storage of reserve medical supplies and other property;
- integration of information technology and telemedicine into JMT operations;
- formalization of accounting, reporting, and notification systems.

CONCLUSION

The conducted research confirms the significance of FMBA JMTs as one of the key components of the overall medical support system in emergency situations, terrorist acts, and armed conflicts. Their practical application, especially during the SMO, demonstrated not only their ability to promptly respond and effectively provide medical care and perform medical evacuation for victims, the wounded, and sick individuals, but also the need for systemic improvement of normative regulation and methodological support for their activities.

The analysis of the normative and methodological framework of JMTs revealed certain discrepancies between its current state and modern requirements. Specifically, there is a lack of clear regulation regarding the creation, preparation, application, and functioning of medical teams; ambiguity in task distribution and DMC functions concerning JMTs; and virtually no normative support for medical evacuation — a key element of JMT operations. Based on the data obtained, the necessity for a comprehensive improvement of the legal and methodological foundation of JMT activities has been substantiated. This entails the development and approval of a uniform Model Regulation for consolidated medical teams, amendments to FMBA Order No. 126 dated 25.04.2022, incorporating tasks related to medical evacuation, assigning DMCs responsibility for forming, preparing, and managing JMTs, and developing detailed methodological recommendations standardizing organizational structure, equipment, and readiness criteria for JMTs.

Implementation of these proposals can enhance the timeliness, efficiency, and resilience of the medical support system for victims, the wounded, and sick individuals. It will create conditions for more adaptive and effective operations of JMTs within a unified modern system countering threats to the security of the Russian Federation.

[13] FMBA medical teams provided medical assistance to 143,000 servicemen in the SMO zone. Kommersant; 2024. URL: https://www.kommersant.ru/doc/7693365 (request date: 08.08.2025).
[14] Order of FMBA of Russia No. 208 "On Establishment of Network of Regional Medical Centers of FMBA of Russia" dated 30.07.2020.
[15] Order of FMBA of Russia No. 126 "On Approval of Cases and Procedures for Providing Medical and Sanitary Care, Specialized, Including High-Tech Medical Care, by Healthcare Professionals of Medical Facilities Subordinated to FMBA of Russia Outside These Facilities" dated 25.04.2022.

Литература / References

1. Goncharov SF, Akinshin AV, Bobiy BV. Organization of Medical Care for Victims of Emergencies in Medical Treatment Organizations of the Federal Medical and Biological Agency. Message 1. *Disaster Medicine*. 2022;4:5–12 (In Russ.). https://doi.org/10.33266/2070-1004-2022-4-5-12
2. Goncharov SF, Akinshin AV, Markov SV, Bobiy BV, Baranova NN. Organization of Medical Care for Victims of Emergencies in Medical Organizations of the Federal Medical and Biological Agency. Message 2. *Disaster Medicine*. 2023;3:5–12 (In Russ.). https://doi.org/10.33266/2070-1004-2023-3-5-12
3. Goncharov SF, Akin'shin AV, Titov IG, Bobij BV. On the Issue of Creating the Disaster Medicine Service of the FMBA of Russia. *A.I. Burnasyan Federal Medical Biophysical Center Clinical Bulletin*. 2022;2:17–25 (In Russ.). https://doi.org/10.33266/2782-6430-2022-2-17-25
4. Akinshin AV, Bobiy BV. Regulation and Methodological Support of Activities of Functional Subsystem of Federal Medical and Biological Agency. *Disaster Medicine*. 2023;2:5–11 (In Russ.). https://doi.org/10.33266/2070-1004-2023-2-5-11
5. Goncharov SF, Markov SV, Cheplyaev AA, Demenko VV, Minok MN, Baranova NN, et al. Center for Medical and Evacuation Support of State Research Center — Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency: Status and Development Prospects. *Disaster Medicine.* 2024;2:21–33 (In Russ.). https://doi.org/10.33266/2070-1004-2024-1
6. Markov SV. The experience of a multi-specialty field hospital as a stage of medical evacuation in the system of medical evacuation support for an armed conflict. *Disaster Medicine*. 2025;1:5–11 (In Russ.). https://doi.org/10.33266/2070-1004-2025-1
7. Agapov VK, Pavlov NB, Shutov AA. Medical care — the major element in emergency relief operations in peacetime. *Extreme Medicine.* 2015;53(3):8–12 (In Russ.).
8. Akhmetova AI, Kukhareva AR, Antonov SI. Flood management measures in Russian far Easten Federal District. *Current issues of modern medicine and healthcare.* Collection of materials of the 6th International (76th Russian) scientific and practical conference. Yekaterinburg; 2021 (In Russ.). EDN: TIUHCG
9. Melnikov GYa. Participation of Clinical Hospital No. 51 FMBA of Russia in the Elimination of the Consequences of the Accident at Sayano-Shushenskaya HPP. *Bulletin of Clinical Hospital No. 51.* 2009;51:6–7 (In Russ.).
10. Gulin AN, Grebenyuk BV. Liquidation of medical and sanitary consequences of flood in Krasnodar territory. *Disaster Medicine.* 2012;3(79):14–5 (In Russ.).
11. Ovcharenko AP, Lemeshkin RN, Tolstosheev VN, Luchshev AV. Analysis of the Experience of Work to Elimination of Medical-Sanitary Consequences of Emergency Situations Provided by Specialists of Unregular Formers of Service of Disaster Medicine of the Ministry of Health of Russia and Federal Medical and Biological Agency of Russia. *Disaster Medicine.* 2023;1:9–13 (In Russ.). https://doi.org/10.33266/2070-1004-2023-1-9-13
12. Medik VA. Ways to improve organizational and methodological activities in medical organizations at the present stage of healthcare system reform. *Bulletin of VSHOUZ.* 2021;7(4):40–50 (In Russ.). https://doi.org/10.33029/2411-8621-2021-7-4-40-50
13. Bobiy BV. Normative Legal Regulation and Organizational and Methodological Support for the Functioning of the Disaster Medicine Service of the Ministry of Health of Russia: Status and Some Ways to Improve Them. *Disaster Medicine.* 2024;4:59–69 (In Russ.). https://doi.org/10.33266/2070-1004-2024-4
14. Goncharov SF, Bystrov MV, Baranova NN, Guseva OI, Popov VP, Romanov VV, et al. Mobile medical formations of service for disaster medicine of Ministry of Health of Russian Federation. *Disaster Medicine.* 2019;3:5–11 (In Russ.). https://doi.org/10.33266/2070-1004-2019-3
15. Sboev AO. Mobile medical unit as a rational model of modern mobile medical formation operating in the metropolis in emergency situations. *Research'n Practical Medicine Journal.* 2020;7(1):83–95 (In Russ.). https://doi.org/10.17709/2409-2231-2020-7-1-9
16. Aleksanin SS, Rybnikov VY, Evdokimov VI, Gudz YV, Bashinskii OA. Methodological aspects of creating mobile medical teams at Russian EMERCOM for Mitigating consequences of emergency situations. *Human Ecology.* 2017;24(11):3–9 (In Russ.). https://doi.org/10.33396/1728-0869-2017-11-3-9

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APPLICATION OF MODIFIED PACKING IN THE COMBINATION TREATMENT OF EXTENSIVE TRAUMATIC LIVER INJURIES

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Introduction. Traumatic liver injuries are a significant challenge in modern emergency surgery. Developing new methods for surgical hemostasis of traumatic liver injuries is an extremely relevant task in urgent surgery.
Objective. Development and testing of surgical hemostasis for traumatic liver injuries using a modified packing technique.
Materials and methods. A new method of surgical hemostasis for traumatic liver injuries was developed using cadaveric material. The clinical trial included 27 patients with severe traumatic liver injuries (AAST Grade IV). Patients were divided into the main (n = 14) and control (n = 13) groups. Patients in the main group were treated using a new method of surgical hemostasis for traumatic liver injuries, involving tamponade of the liver wounds with Surgitamp hemostatic gauze impregnated with Molselect G-50 granular sorbent, followed by modified liver packing with strips of polypropylene mesh implant. In the control group, surgical hemostasis was performed by tamponading the liver wound with hemostatic sponges followed by suturing. The effectiveness of the proposed method was evaluated based on the following parameters: definitive hemostasis, number of recurrent hemorrhages, number of recurrent operations, mortality, hospital stay duration, intensive care unit stay duration. Statistical analysis of the study results was performed using the Statistica 10 software.
Results. The application of modified packing in the combination treatment of patients with severe traumatic liver injuries increased the reliability of definitive hemostasis from 46.2% to 92.8% (p = 0.0391), reduced the number of re-bleeding episodes and re-operations from 38.4% to 7.1% (p = 0.0391), and decreased the mortality rate from 38.4% to 14.2% (p > 0.05).
Conclusions. The application of the new combined method of surgical hemostasis improved the treatment outcomes for patients with severe traumatic liver injuries by increasing the reliability of definitive hemostasis, reducing re-bleeding and re-operations, and lowering mortality.

Keywords: abdominal trauma; liver injuries; intra-abdominal bleeding; surgical hemostasis; Surgitamp; Molselect G-50; modified packing

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ПРИМЕНЕНИЕ МОДИФИЦИРОВАННОГО ПАКЕТИРОВАНИЯ В КОМПЛЕКСНОМ ЛЕЧЕНИИ ОБШИРНЫХ ТРАВМАТИЧЕСКИХ ПОВРЕЖДЕНИЙ ПЕЧЕНИ

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Введение. Травматические повреждения печени представляют собой серьезную проблему современной ургентной хирургии. Разработка новых методов хирургического гемостаза травматических повреждений печени является крайне актуальной задачей неотложной хирургии.
Цель. Разработка и апробация хирургического гемостаза при травматических повреждениях печени путем применения модифицированного пакетирования.
Материалы и методы. На кадаверном материале разработан новый метод хирургического гемостаза при травматических повреждениях печени. В клиническом исследовании приняли участие 27 пациентов с тяжелыми травматическими повреждениями печени (IV степень по AAST). Пациенты были разделены на две группы: основную (n = 14) и контрольную (n = 13). В лечении пациентов основной группы применен новый способ хирургического гемостаза травматических повреждений печени, заключающийся в тампонировании ран печени гемостатической марлей «Сургитамп», пропитанной гранулированным сорбентом «Молселект G-50», с последующим модифицированным пакетированием печени полосками полипропиленового сетчатого импланта; в контрольной группе хирургический гемостаз выполняли путем тампонирования раны печени гемостатическими губками с последующим ушиванием раны. Эффективность применения методики оценивали по следующим показателям: окончательный гемостаз, количество повторных кровотечений, количество повторных операций, летальность, длительность стационарного лечения, длительность пребывания в ОРИТ. Статистическую обработку результатов исследования проводили в программе Statistica 10.

Результаты. Применение модифицированного пакетирования в комплексном лечении пациентов с тяжелыми травматическими повреждениями печени позволило повысить надежность окончательного гемостаза с 46,2 до 92,8% (p = 0,0391), снизить количество повторных кровотечений и операций с 38,4 до 7,1% (p = 0,0391) и уменьшить уровень летальности с 38,4 до 14,2% (p > 0,05).
Выводы. Применение нового комбинированного метода хирургического гемостаза позволило улучшить результаты лечения пациентов с тяжелыми травматическими повреждениями печени за счет повышения надежности окончательного гемостаза, уменьшения повторных кровотечений и операций, снижения летальности.

Ключевые слова: абдоминальная травма; повреждения печени; внутрибрюшное кровотечение; хирургический гемостаз; Сургитамп; Молселект G-50; модифицированное пакетирование

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование проведено с соблюдением требований и норм Хельсинкской декларации. Исследование одобрено этическим комитетом ФГБОУ ВО ВГМУ им. Н.Н. Бурденко (протокол № 2 от 05.04.2022). Все пациенты подписали информированное согласие на лечение, а также использование обезличенных медицинских данных и фотографий. Кадаверный материал предоставлен бюджетным учреждением здравоохранения Воронежской области «Воронежское областное патолого-анатомическое бюро». Разрешение на предоставление кадаверного материала соответствовало положениям действующего законодательства.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Traumatic injuries to the abdominal organs represent a serious problem in modern emergency surgery. According to statistics, abdominal trauma accounts for 1.5–36.5% of all traumatic injuries. However, the mortality rate for injuries to the abdominal organs reaches extremely high figures — approximately 60%. Furthermore, the majority of the victims are of young and working age, which underscores the significant medical and social importance of this problem [1, 2].

A distinct category of injuries in abdominal trauma comprises those to the parenchymal organs. Damage to the spleen is most frequently encountered in clinical practice. Liver injuries rank second in the structure of abdominal parenchymal organ injuries, yet they represent a considerable challenge for global surgery. Severe liver injuries are often accompanied by the development of difficult-to-control intra-abdominal hemorrhage and the onset of hepatocellular insufficiency in the postoperative period in cases of massive liver crush injury, which are the primary causes of unsatisfactory treatment outcomes for victims with liver trauma and high mortality rates (up to 50% or more) in such injuries [3–5].

Most recent studies concerning the problem of traumatic liver injuries have been focused on refining tactical approaches to the management of liver trauma, the application of endovascular hemostasis technologies, and the improvement of methods for surgical hemostasis of liver injuries [6]. Among the tactical approaches for the management of severe combined injuries, the strategy of staged surgical treatment, so called “damage control surgery,” is gaining increasing adoption. This strategy aims to shorten the initial phase of operative intervention, followed by stabilization of the patient’s condition and performance of the definitive surgical stage under more favorable circumstances. The strategy of damage control surgery currently demonstrates favorable outcomes; however, it does not address all aspects of the surgical management of severe traumatic liver injuries due to the development of early recurrent hemorrhages in patients with massive hepatic parenchymal damage [7–9].

An important modern achievement in the management of abdominal trauma, and primarily intra-abdominal hemorrhages, has been the adoption of the Non-Operative Management (NOM) strategy [10, 11]. The NOM strategy implies conducting a strict patient selection process and equipping hospitals with angiographic suites, coupled with a mandatory system for dynamic monitoring of patient condition [12]. However, most Russian hospitals providing emergency care to patients with abdominal trauma lack the appropriate facilities, which limits the applicability of the NOM strategy in the treatment of patients with liver injuries [13].

The primary current method for the management of severe traumatic liver injuries in patients with unstable hemodynamics is emergency operative intervention — midline laparotomy. Its main objective is to achieve reliable hemostasis of intra-abdominal hemorrhage [14, 15]. A multitude of methods for hemostasis of liver injuries, both temporary and definitive, have been developed and are used in clinical practice; however, none of them can be considered fully satisfactory due to the high incidence of recurrent hemorrhage in the postoperative period [16, 17]. The development of new methods for surgical hemostasis of traumatic liver injuries remains an extremely urgent task in modern emergency surgery [18].

In this study, we set out to develop and test a surgical hemostasis method for traumatic liver injuries through the application of modified perihepatic packing.

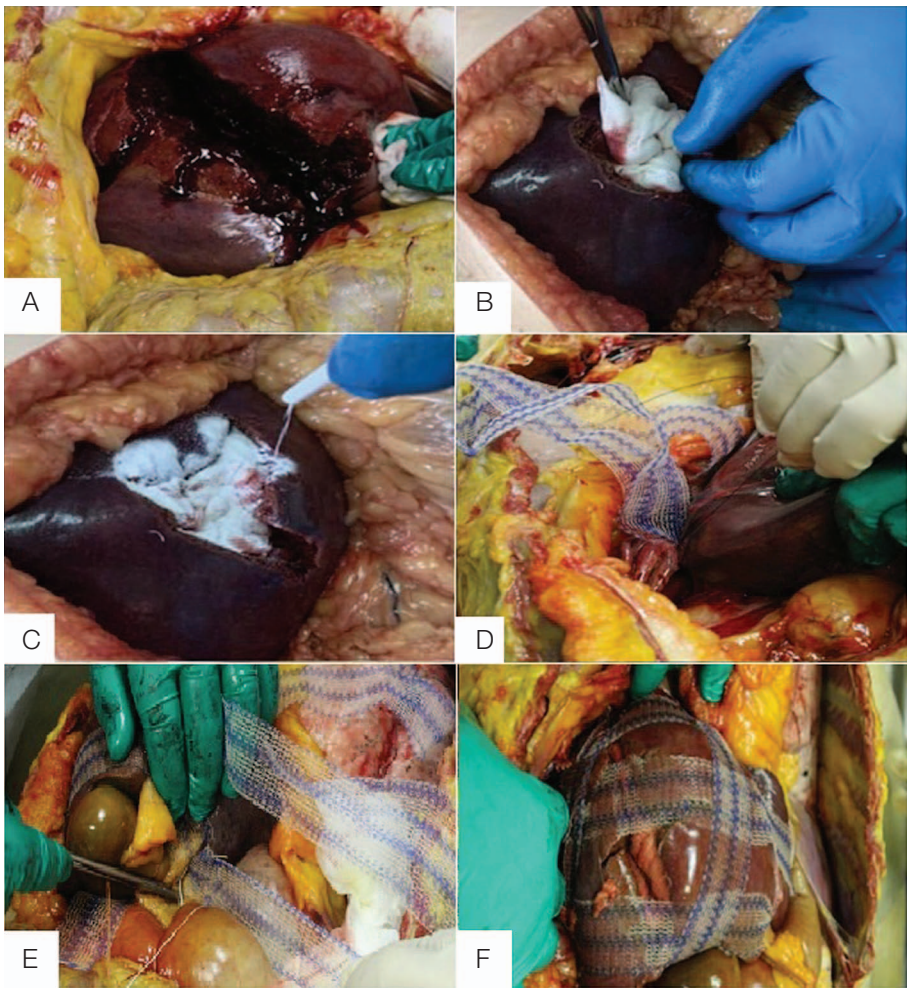
MATERIALS AND METHODS

Earlier, we conducted preliminary experimental studies to investigate the feasibility of a modified perihepatic packing technique for surgical hemostasis in laboratory animals [19].

In addition, we conducted preliminary studies on cadaveric material to develop a surgical technique for the application of modified perihepatic packing. The studies were carried out on seven unfixed male cadavers with a mean age of 34.5 ± 3.2 years, who had died from causes unrelated to traumatic injuries or diseases of the abdominal organs. The cadavers were provided by the Voronezh Regional Pathological Anatomy Bureau. The authorization for the provision of the cadaveric material complied with the provisions of the current legislation.[1] The time interval from death to the study procedure did not exceed 24 h.

In each research subject, a traumatic liver injury was simulated. To that end, following a total laparotomy, the right lobe of the liver was exteriorized into the surgical wound and placed on a supporting platform/table.

At a distance of 40 cm from the liver surface, a 250 g steel weight was suspended from a laboratory stand using a thread. Upon readiness, the thread securing the weight was burned through with a gas burner flame, causing the weight to fall vertically and impact the diaphragmatic surface of the right liver lobe, resulting in the formation of a liver wound (Fig. 1A). The simulated liver wound was tightly packed with Surgitamp



Photographs taken by the authors

Fig. 1. Stages of modified perihepatic packing on a cadaveric model: A — simulated contused wound of the right liver lobe; B — tamponade of the liver wound with Surgitamp hemostatic material; C — application of Molselect G-50 granular sorbent; D — fixation of the polypropylene mesh implant strips to the diaphragm; E — fixation of the mesh implant strips along the visceral surface of the liver; F — final appearance of the liver after modified perihepatic packing

[1] Decree of the Government of the Russian Federation No. 750 "On the Approval of the Rules for the Transfer of Unclaimed Bodies, Organs, and Tissues of a Deceased Person for Use in Medical, Scientific, and Educational Purposes, as well as the Use of Unclaimed Bodies, Organs, and Tissues of a Deceased Person for the Said Purposes" (as amended) of 21.07.2012.

hemostatic gauze (Fig. 1B), which had been impregnated with Molselect G-50 granular sorbent (Fig. 1C), after which the right liver lobe was compressed with gauze pads.

To perform the procedure of modified perihepatic packing, mobilization of the right liver lobe was carried out. To that end, the right triangular, coronary, falciform, and round ligaments of the liver were transected with scissors, after which the right liver lobe was exteriorized into the surgical wound. Subsequently, a strip (width 4.0 cm and length 30.0 cm) of a mesh polypropylene implant used for hernia repair was positioned in the sagittal plane. For this purpose, the edge of the mesh implant strip was sutured to the diaphragm with three separate interrupted sutures using polypropylene monofilament (Fig. 1D). Next, the diaphragmatic surface of the liver was wrapped, extending onto the visceral surface; the mesh implant was tensioned to provide compression, and its lower edge was fixed with interrupted sutures to the starting edge of the strip, creating compression and tension. It is important to perform the modified perihepatic packing by maintaining a distance of 1.0–1.5 cm from the edges of the gallbladder; thereafter, it was enveloped with the greater omentum (Fig. 1E).

To provide additional compression in the transverse direction relative to the right liver lobe, three additional polypropylene mesh strips were sutured in place (Fig. 1F). The strips were enveloped with the greater omentum, which was then fixed to the liver capsule with interrupted sutures to prevent the occurrence of intestinal fistulas.

The subsequent phase of the study comprised the clinical evaluation of the modified perihepatic packing technique in patients with liver injuries. A pilot randomized controlled clinical trial was conducted at Bryansk City Hospital No. 1, Voronezh Regional Clinical Hospital No. 1, Voronezh City Emergency Clinical Hospital No. 1, and Voronezh City Emergency Clinical Hospital No. 10. In total, 27 patients with traumatic liver injury were enrolled in the study. The clinical trial design is summarized in Figure 2.

The inclusion criteria for the study were blunt abdominal trauma; patient age 18–80 years; signed informed consent for participation in the clinical trial; Grade IV liver injury according to the American Association for the Surgery of Trauma (AAST) classification [19].

The exclusion criteria for the study were refusal to participate in the clinical trial; moribund state of the patient; allergic reactions to Molselect G-50, Surgitamp, or the polypropylene mesh implant.

The discontinuation criteria for the study were withdrawal of consent for continued participation in the clinical trial; injury to hollow organs, peritonitis; Grade I–III liver injury according to the AAST classification; multiple injuries to abdominal organs; macroscopic signs of liver cirrhosis; a score of > 13 points on the Military Field Surgery scale for determining surgical tactics.

According to the formulated objectives, the patients were divided into two groups: the main group (14 patients) and the control group (13 patients). Patient allocation was performed by random sampling using a random number generator. The patients were comparable in terms of sex, age, nature of traumatic liver injury, structure of associated injuries, and severity of blood loss.

In the main group, the method for surgical hemostasis of traumatic liver injuries, as described in the patent [20], was applied. In the control group, the method for surgical hemostasis of liver wounds involved tamponade of the liver wounds with hemostatic collagen sponges, followed by suturing of the liver wound with 2-0 PGA monofilament.

As part of combined management, patients in both groups received standard conservative treatment in the postoperative period, which included hemostatic and infusion therapy, blood transfusions as indicated, antibiotic therapy, analgesics, and wound dressings.

The study outcomes were assessed based on the following parameters: achievement of definitive hemostasis, number of recurrent hemorrhages and reoperations, operative time, length of stay in the intensive care unit, duration of hospital stay, mortality rate, and the incidence and nature of postoperative complications.

Statistical analysis of the study results was performed using the Statistica 10 software. Standard descriptive statistics were calculated (mean (M), standard error of the mean (m), median (M_e), lower (Q_1) and upper (Q_3) quartiles). The Shapiro–Wilk test was used to assess the normality of distribution for quantitative variables. The significance of differences for quantitative variables between independent groups was determined using Student's t-test (for normally distributed variables) and the Mann–Whitney U-test (for non-normally distributed variables). Analysis of qualitative variables was conducted using Fisher's exact test and the Z-test with Yates' correction. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The general characteristics of the patients enrolled in the study are presented in Table 1.

Table 1 summarized data on the patient groups. Traumatic liver injuries were most frequently encountered in males of working age — 20 (74.1%). These injuries were significantly less common in females — 7 (25.9%) patients. The median age of patients with traumatic liver injuries was 36.0 [30.0; 43.0] years. Analyzing the mechanism of abdominal trauma, it should be noted that road traffic accidents were the predominant cause of traumatic liver injuries (12 (44.5%) patients), followed by catatrauma (fall from height) (9 (33.3%) patients) and domestic injury (6 (22.2%) patients).

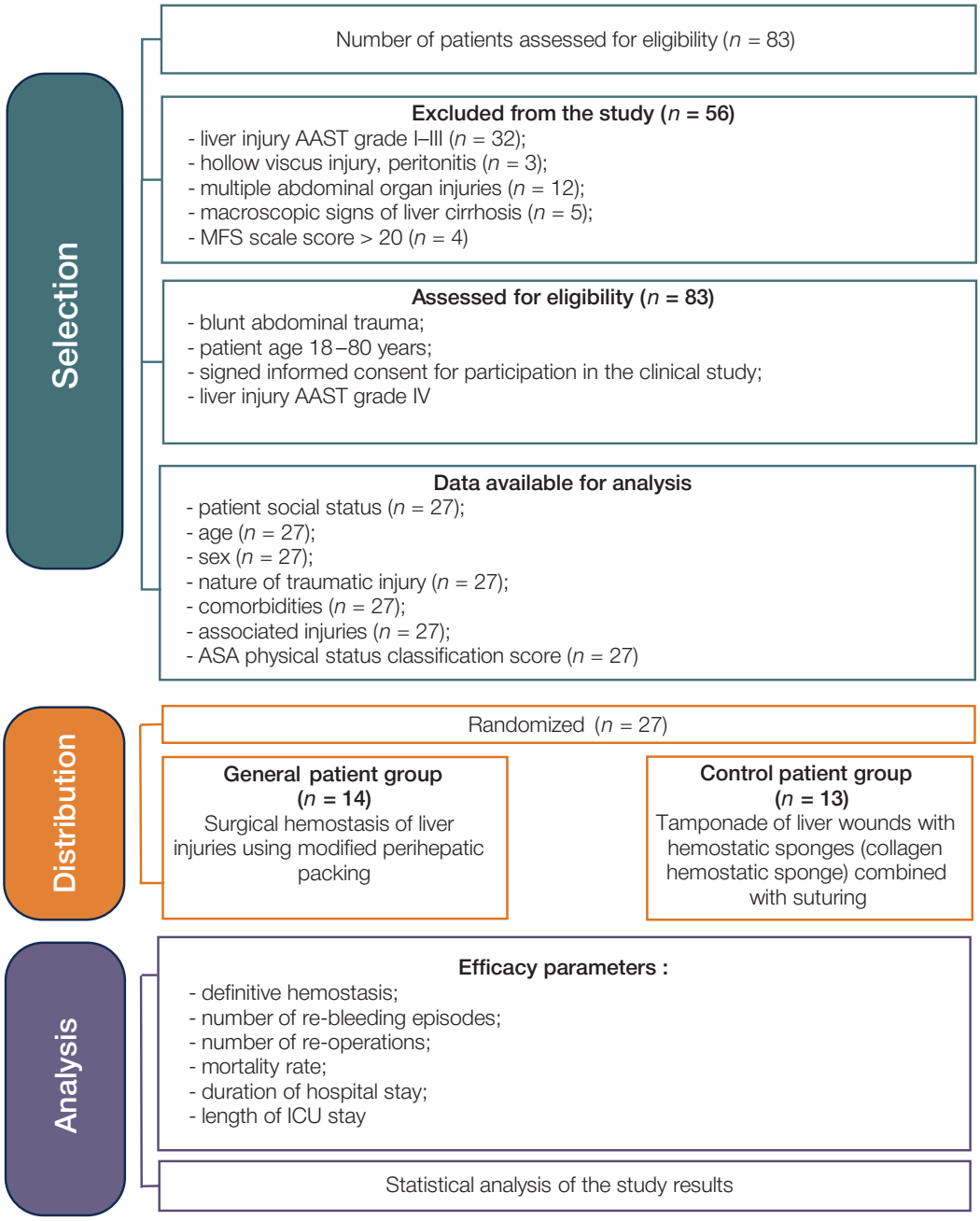


Figure prepared by the authors

Fig. 2. Clinical study design: MFS — Military Field Surgery; ICU — Intensive Care Unit; ASA — American Society of Anesthesiologists; AAST — American Association for the Surgery of Trauma

Among the associated injuries, chest injuries were the most frequent — 17 (32.5%), followed by skeletal trauma — 9 (16.7%), traumatic brain injury — 8 (29.6%), and pelvic injury — 5 (18.5%).

An analysis of comorbidities revealed that patients with abdominal trauma and liver injuries were largely somatically healthy. Comorbid conditions were identified in only 4 (14.8%) patients: coronary artery disease was present in 1 (3.7%) patient, and chronic pancreatitis was found in 3 (11.1%) patients.

As part of combined management of patients in the main group, a new method for surgical hemostasis of traumatic liver injuries was applied. This method involved tamponade of the liver wounds with Surgitamp hemostatic gauze impregnated with Molselect G-50 granular sorbent, followed by modified perihepatic packing using strips of a polypropylene mesh implant [20] (Fig. 3A–F).

We established that immediately after tamponade of the liver wound with Surgitamp hemostatic gauze impregnated with Molselect G-50 granular sorbent, which swells upon contact with blood and increases in volume, the sorbent transformed into a blood-soaked hydrogel. The Surgitamp hemostatic gauze provided compression and enhanced the hemostatic effect. Manual

Table 1. Characteristics of the study groups

Parameter	Main group (n = 14)	Control group (n = 13)	Statistical significance level, p
Currently employed, n (%)	3 (21.4%)	5 (38.5%)	$p > 0.05$
Unemployed, n (%)	8 (57.2%)	8 (61.5%)	$p > 0.05$
Retirees, n (%)	3 (21.4%)	0	id
Age, years $M_e[Q_1; Q_3]$	40 [54.0; 79.0]	36.7 [37.0; 61.0]	$p > 0.05$
Males, n (%)	10 (58.7%)	10 (78.2%)	$p > 0.05$
Females, n (%)	4 (41.3%)	3 (21.8%)	$p > 0.05$
Road traffic injury, n (%)	5 (35.7%)	7 (53.8%)	$p > 0.05$
Fall from height, n (%)	5 (21.4%)	4 (23.1%)	$p > 0.05$
Domestic injury, n (%)	4 (28.6%)	2 (15.4%)	$p > 0.05$
Coronary artery disease, n (%)	1 (83.2%)	0	id
Chronic pancreatitis, n (%)	1 (8.4%)	2 (9.0%)	$p > 0.05$
Traumatic brain injury, n (%)	4 (60.5%)	4 (50.0%)	$p > 0.05$
Chest injuries, n (%)	9 (30.9%)	8 (41.6%)	$p > 0.05$
Skeletal trauma, n (%)	5 (8.6%)	4 (8.4%)	$p > 0.05$
Pelvic bone injury, n (%)	3 (21.4%)	2 (15.3%)	$p > 0.05$
II, n (%)	5 (35.7%)	4 (30.8%)	$p > 0.05$
III, n (%)	7 (50%)	8 (61.5%)	$p > 0.05$
IV, n (%)	2 (14.3%)	1 (7.7%)	$p > 0.05$
III, n (%)	4 (28.6%)	3 (23.2%)	$p > 0.05$
IV, n (%)	6 (42.8%)	5 (38.4%)	$p > 0.05$
V, n (%)	4 (28.6%)	5 (38.4%)	$p > 0.05$

Table compiled by the authors based on original data

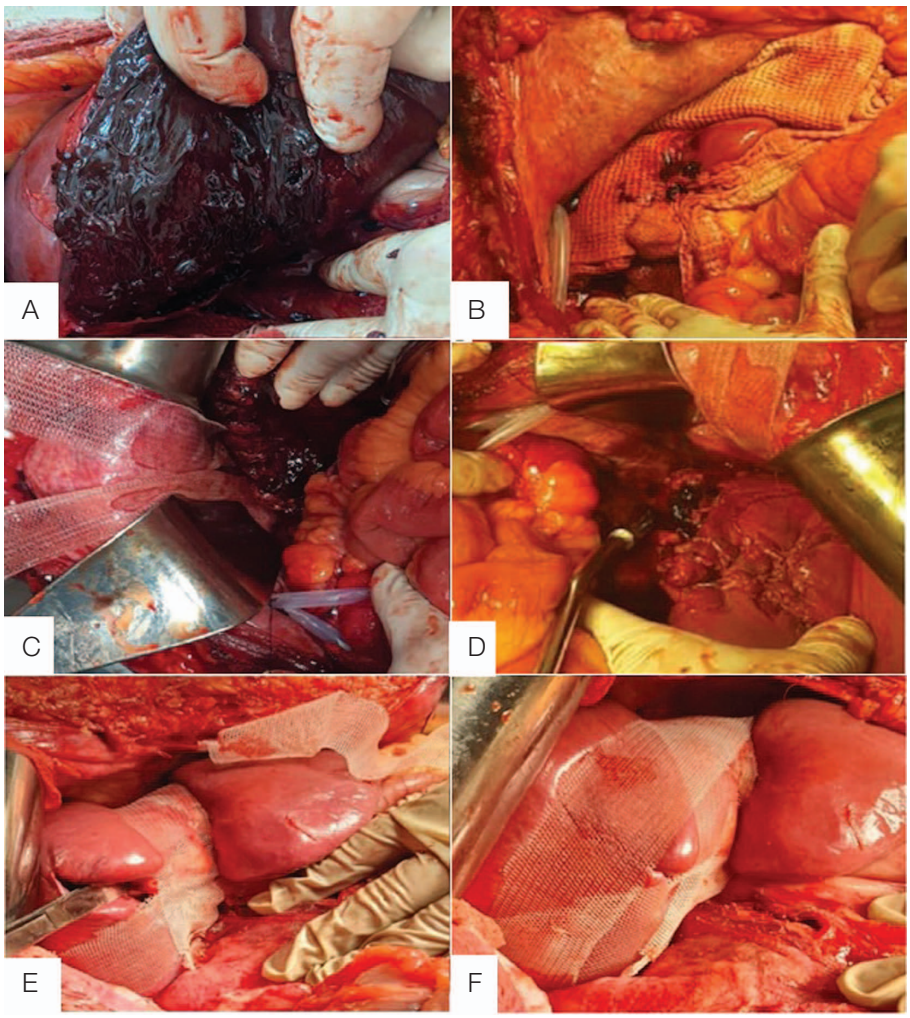
Note: id — insufficient data; n — patient number in the group.

compression of the liver achieved temporary hemostasis; however, upon release of compression, the bleeding recurred.

To achieve definitive hemostasis, the method of modified perihepatic packing with strips of polypropylene mesh implant was employed (the fixation technique is described above), which resulted in the final cessation of bleeding. Definitive hemostasis was achieved in 13 (92.8%) patients in the main group. In one (7.14%) patient from the main group, signs of recurrent hemorrhage were observed on postoperative day 2. The patient underwent emergency reoperation. During the abdominal cavity revision, the source of bleeding was identified as diffuse oozing from the liver wound due to insufficient compression from the tension of the polypropylene mesh implant strips. Additional tightening of the strips led to hemostasis and achievement of definitive control.

In the main group, two (14.3%) patients died. The cause of death was severe polytrauma. According to forensic autopsy findings, no signs of recurrent intra-abdominal hemorrhage were observed in either case.

In the control group, to achieve definitive hemostasis of the liver injuries, the wounds were packed with hemostatic collagen sponges. The sponges became saturated with blood; however, diffuse bleeding from the liver wounds persisted. To achieve definitive hemostasis, the liver wounds were sutured with U-shaped stitches (using 2-0 PGA monofilament) to approximate the wound edges. During suturing of the liver wounds, diffuse oozing from the needle puncture sites was observed, and the



Photographs taken by the authors

Fig. 3. Stages of modified perihepatic packing in a patient with liver injury: A — gross appearance of a traumatic rupture of segments V, VI, and VII of the right liver lobe (AAST Grade IV); B — tamponade of the wound with Surgitamp hemostatic material impregnated with Molselect G-50 granular sorbent, and temporary packing with a gauze towel; C — fixation of the polypropylene mesh implant strips to the diaphragm; D, E — fixation of the mesh implant strips in the transverse direction; F — final appearance of the surgical wound

liver parenchyma was lacerated by the suture material, which complicated the achievement of definitive hemostasis and prolonged the operative time. In five (38.4%) patients in the control group, signs of recurrent bleeding were observed on postoperative days 2–3. These patients underwent emergency reoperation. The source of bleeding in all cases was oozing from the liver ruptures. In the control group, five (38.4%) patients died due to acute blood loss, posthemorrhagic anemia, and associated injuries.

The comparative effectiveness of modified perihepatic packing in the management of traumatic liver injuries is presented in Table 2.

The application of modified perihepatic packing in the combination management of patients with severe traumatic liver injuries (AAST Grade IV) resulted in an increase in the reliability of definitive hemostasis from 46.2% to 92.8% (p = 0.0391), a reduction in the rate of recurrent hemorrhages and reoperations from 38.4% to 7.1% (p = 0.0391), and a decrease in mortality from 38.4% to 14.2% ($p > 0.05$).

The conducted analysis of postoperative surgical complications in the study groups showed that in the postoperative period, one patient in the main group experienced recurrent hemorrhage. The patient underwent emergency reoperation. During the abdominal cavity revision, the source of bleeding was identified as diffuse oozing from the liver wound due to insufficient compression from the tension of the polypropylene mesh implant strips. Additional tightening of the strips led to the cessation of bleeding and the achievement of definitive hemostasis.

In the control group, five patients exhibited signs of recurrent bleeding in the postoperative period. These patients underwent reoperation. Intraoperatively, laceration of the liver parenchyma by the sutures was identified;

Table 2. Comparative effectiveness of management for patients with traumatic liver injuries

Parameter	Main group (*n* = 14)	Control group (*n* = 13)	Statistical significance level, *p*
Definitive hemostasis, *n* (%)	13 (92.8%)	6 (46.2%)	$p = 0.0391$
Recurrent hemorrhages, *n* (%)	1 (7.1%)	5 (38.4%)	$p = 0.0391$
Reoperations, *n* (%)	1 (7.1%)	5 (38.4%)	$p = 0.0391$
Mortality, *n* (%)	2 (14.2%)	5 (38.4%)	$p > 0.05$
Median duration of hospital stay, bed days	10.0 [9.0; 14.0]	16.0 [10.0; 18.0]	$p > 0.05$
Median operative time, min	100.0 [85.0; 120.0]	130.0 [120.0; 150.0]	$p = 0.0022$
Median ICU length of stay, bed-days	3.0 [1.0; 4.0]	5.0 [3.0; 6.0]	$p = 0.011$
Median volume of red blood cell transfusion, mL	1140 [1100; 1200]	1230 [1150; 1900]	$p = 0.0272$
Median volume of FFP transfusion, mL	1175 [1100; 1250]	1430 [1350; 2100]	$p = 0.0001$
Postoperative complications (according to the Clavien–Dindo classification)			
III, *n* (%)	1 (7.1%)	5 (38.4%)	$p > 0.05$
IIIa, *n* (%)	–	1 (7.7%)	id
IV, *n* (%)	–	1 (7.7%)	id
V, *n* (%)	2 (14.2%)	5 (38.4%)	$p > 0.05$

Table compiled by the authors based on original data

Note: ICU — Intensive Care Unit; FFP — Fresh Frozen Plasma; id — insufficient data; *n* — patient number.

additional hemostasis was performed, comprising electrocautery, application of a Tachocomb® patch to the site of diffuse oozing, or additional suturing of the rupture sites. One patient in the control group developed complaints of right upper quadrant pain and weakness in the postoperative period; an abdominal ultrasound was performed, revealing a biloma in the abdominal cavity. The latter complication required ultrasound-guided percutaneous drainage. The outcome was complete resolution, and the patient was discharged in satisfactory condition for outpatient follow-up on day 18. One patient developed disseminated intravascular coagulation (DIC) syndrome in the postoperative period following acute massive blood loss, which required intensive care in the ICU; this complication resolved.

DISCUSSION

Injuries to the abdominal organs represent one of the most challenging and largely unresolved problems in emergency surgery [5, 9]. In the spectrum of abdominal trauma, liver injuries rank second, surpassed only by splenic injuries. However, the highest mortality rates (14–58%) in patients with blunt abdominal trauma are associated precisely with severe liver injuries, which is attributable to massive, difficult-to-control intra-abdominal hemorrhage and the development of hepatocellular insufficiency in the postoperative period [13, 18].

The most frequent cause of mortality in abdominal trauma is traumatic liver rupture complicated by massive hemorrhage. In such cases, two factors are of paramount importance: the promptness and rapidity of surgical intervention and the application of a reliable and effective method for surgical hemostasis [6].

Most surgeons in emergency settings employ wound suturing, electrocautery, and packing. A significant drawback of these methods is the development of ischemic necrotic areas between the sutures. For deep injuries, packing the wounds with omentum, various hemostatic sponges, films, or powdered hemostatic agents is used. However, as practice shows, these often prove to be ineffective. Tamponade of deep wounds with a towel, gauze pad, or any non-resorbable material necessitates reoperation [3].

In cases of extensive traumatic liver injuries, an effective method for hemorrhage control is perihepatic packing of the liver ruptures with a polypropylene mesh implant. This procedure involves mobilization of the liver from all its ligaments while mandatorily preserving venous outflow. However, in the postoperative period, after stabilization of all patients, reoperation is required to perform definitive surgical repair [1].

The presented data indicate that while numerous methods for hemostasis in liver injuries exist, none of them is fully satisfactory. In this context, the development of new methods for surgical hemostasis of traumatic liver injuries remains a relevant problem in emergency surgery.

Prior to the initiation of the clinical trial, we conducted multifunctional integrated morphological, morphometric, and immunohistochemical studies in laboratory animals to develop a new method for surgical hemostasis [3]. In a series of *in vivo* experiments, it was initially demonstrated that the application of Surgitamp hemostatic agent and Molselect G-50 sorbent effectively controls moderate parenchymal bleeding in models of bleeding liver wounds. Furthermore, in experiments using models with active hemorrhage from liver wounds, the developed technique of combined surgical hemostasis — utilizing hemostatic agents and modified perihepatic packing — demonstrated high efficacy in achieving local hemostasis [19].

In accordance with the aim of our clinical study, the newly developed method of surgical hemostasis with modified perihepatic packing [20] was applied to the most severely affected category of patients with traumatic liver injury complicated by active hemorrhage (AAST Grade IV).

CONCLUSION

A new method for surgical hemostasis has been developed and introduced into clinical practice. This method involves tamponade of liver wounds with Surgitamp hemostatic gauze impregnated with Molselect G-50 granular sorbent, followed by modified perihepatic packing using strips of a polypropylene mesh endoprosthesis. This approach has improved treatment outcomes for patients with severe traumatic liver injuries by enhancing the reliability of definitive hemostasis, reducing the incidence of recurrent hemorrhages and reoperations, and lowering the mortality rate.

References

1. García IC, Villalba JS, Iovino D, Franchi C, Iori V, Pettinato G, et al. Liver Trauma: Until When We Have to Delay Surgery? A Review. *Life*. 2022;12(5):694. https://doi.org/10.3390/life12050694
2. Corvino F, Giurazza F, Marra P, Ierardi AM, Corvino A, Basile A, et al. Damage Control Interventional Radiology in Liver Trauma: A Comprehensive Review. *Journal of Personalized Medicine*. 2024;14(4):365. https://doi.org/10.3390/jpm14040365
3. Vorontsov AK, Parkhisenko YuA, Cherednikov EF, Barannikov SV, Chernykh AV, Bezaltynnykh AA, et al. Experimental justification for the use of a new method of surgical hemostasis of extensive traumatic liver injuries. *Volgograd Scientific Medical Journal*. 2023;20(2):45–50 (In Russ.). EDN: HSJMKR
4. Evtikhov AV, Lyubivy ED, Kim VL. Clinical observations of severe traumatic liver injuries. *Pirogov Russian Journal of Surgery*. 2020;7:89–92 (In Russ.). https://doi.org/10.17116/hirurgia202007189
5. Aleksandrov VV, Maskin SS, Alimov MN, Biryulev DS, Matyukhin VV, Rashid A, et al. Closed liver injury. A systematic review. *Russian Sklifosovsky Journal "Emergency Medical Care"*. 2025;14(1):141–54 (In Russ.). https://doi.org/10.23934/2223-9022-2025-14-1-141-154
6. Coccolini F, Coimbra R, Ordonez C, Kluger Y, Vega F, Moore EE, et al. Liver trauma: WSES 2020 guidelines. *World Journal of Emergency Surgery*. 2020;15(1):24. https://doi.org/10.1186/s13017-020-00302-7
7. Benz D, Balogh ZJ. Damage control surgery: current state and future directions. *Current Opinion in Critical Care*. 2017;23(6):491–7. https://doi.org/10.1097/mcc.0000000000000465
8. Ordoñez CA, Parra MW, Millán M, Caicedo Y, Guzman-Rodriguez M, Padilla N, et al. Damage Control in Penetrating Liver Trauma: Fear of the Unknown. *Colombia Médica*. 2020;51(4):e4134365.
9. Chubaryan KA, Groshilin YuV, Lukash VS. Possibilities of improving the results of surgical treatment of patients with severe liver injuries. *Polytrauma*. 2024;3:19–27 (In Russ.). https://doi.org/10.24412/1819-1495-2024-3-19-27
10. Brooks A, Reilly JJ, Hope C, Navarro A, Naess PA, Gaarder C. Evolution of non-operative management of liver trauma. *Trauma Surgery and Acute Care Open*. 2020;5(1):e000551. https://doi.org/10.1136/tsaco-2020-000551
11. Iacobellis F, Di Serafino M, Caruso M, Dell'Aversano Orabona G, Rinaldo C, Grimaldi D, et al. Non-Operative Management of Polytraumatized Patients: Body Imaging beyond CT. *Diagnostics*. 2023;13(7):1347. https://doi.org/10.3390/diagnostics13071347
12. Aleksandrov VV, Maskin SS, Ermolaeva NK, Matyukhin VV. Conservative management of patients with closed trauma of parenchymal organs of the abdominal cavity and retroperitoneal space, with retroperitoneal hemorrhages — indications, methodology and feasibility. *Russian Sklifosovsky Journal "Emergency Medical Care"*. 2021;3:540–7 (In Russ.). https://doi.org/10.23934/2223-9022-2021-10-3-540-548
13. Gavrishchuk YaV, Manukovsky VA, Tulupov AN, Demko AE, Kolchanov EA, Savello VE, et al. Diagnostics and treatment of liver injuries in blunt abdominal trauma using minimally invasive technologies. *Grekov's Bulletin of Surgery*. 2022;181(3):28–36 (In Russ.). https://doi.org/10.24884/0042-4625-2022-181-3-28-36
14. Podoluzhny VI, Radionov IA, Pelts VA, Startsev AB, Krasnov KA, Shatalin VA. Traumatic liver injury. *Polytrauma*. 2023;1:34–8 (In Russ.). https://doi.org/10.24412/1819-1495-2023-1-34-38
15. Lipatov VA, Lazarenko SV, Severinov DA, Denisov AA, Chupakhin EG, Aniskina EN. Comparative analysis of the effectiveness of new samples of local hemostatic agents. *Extreme Medicine*. 2023;25(4):131–6 (In Russ.). https://doi.org/10.47183/mes.2023.063
16. Barannikov SV, Cherednikov EF, Polubkova GV, Banin IN, Bolkhovitinov AE, Berezhnoy SI, et al. Use of alginate polymer polysaccharide hemostatic hydrogel in ongoing arterial bleeding from a peptic ulcer of a gastroenteroanastomosis (clinical case). *Extreme Medicine*. 2025;27(2):235–42 (In Russ.). https://doi.org/10.47183/mes.2025-243
17. Gavrishchuk YaV, Manukovsky VA, Tulupov AN, Demko AE, Kolchanov EA, Kisilev M, et al. Treatment of a victim with closed liver injury using interventional methods. *Bulletin of Experimental*

and Clinical Surgery. 2023;16(4):316–20 (In Russ.). https://doi.org/10.18499/2070-478X-2023-16-4-316-320
18. Tinkoff G, Esposito TJ, Reed J, Kilgo P, Fildes J, Pasquale M, et al. American Association for the Surgery of Trauma Organ Injury Scale I: spleen, liver, and kidney, validation based on the National Trauma Data Bank. *Journal of the American College of Surgeons*. 2008;207(5):646–55. https://doi.org/10.1016/j.jamcollsurg.2008.06.342
19. Vorontsov AK, Parkhisenko YuA, Cherednikov EF, Barannikov SV, Bezaltynnykh AA, Chernykh AV. Hemostatic Gauze Surgitamp and Granular Sorbent Molselect G-50 Application in the Treatment of Simulated Bleeding Liver Wounds. *Journal of Experimental and Clinical Surgery.* 2023;1(16):52–9 (In Russ.). https://doi.org/10.18499/2070-478X-2023-16-1-52-59
20. Vorontsov AK, Parkhisenko YuA, Vorontsov KE, Bezaltynnykh AA. *A method of surgical treatment for patients with severe liver injuries.* Patent of Russian Federation № 2674874 C1; 2018 (In Russ.). EDN: ZECXBR

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REMOVAL OF A METAL FRAGMENT AND FLOATING THROMBUS FROM THE INTERNAL JUGULAR VEIN OF A SERVICEMAN IN A FIELD HOSPITAL

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Introduction. Fragment wounds of the neck with neurovascular bundle injury sustained during combat operations represent a relevant problem in the field of extreme medicine. High mortality rates, along with a lack of sufficient research, contribute to uncertainty in determining optimal treatment tactics.
Case report. A successful surgical treatment of a serviceman with a fragment wound to the neck caused by a foreign metallic body (shell fragment) involving the internal jugular vein and a floating thrombus was performed in a field hospital. An open surgery was conducted to remove the foreign metallic body and the floating thrombus from the internal jugular vein, followed by repair of the venous wall with a 7/0 polypropylene suture. The postoperative course was uneventful.
Conclusions. The presented case demonstrates previously unpublished data on a variant of fragment injury to the internal jugular vein. The proposed surgical technique has proven to be effective and safe.

Keywords: internal jugular vein thrombosis; floating thrombus; fragment wound of the neck; military field surgery; foreign body of the jugular vein; thrombectomy

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УДАЛЕНИЕ МЕТАЛЛИЧЕСКОГО ОСКОЛКА И ФЛОТИРУЮЩЕГО ТРОМБА ИЗ ВНУТРЕННЕЙ ЯРЕМНОЙ ВЕНЫ У ВОЕННОСЛУЖАЩЕГО В ВОЕННО-ПОЛЕВОМ ГОСПИТАЛЕ

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Введение. Осколочные ранения шеи с повреждением сосудисто-нервного пучка в ходе ведения боевых действий являются актуальной проблемой медицины экстремальных ситуаций. Высокая смертность, а также отсутствие достаточного количества исследований создают неопределенность в выборе тактики лечения.
Описание клинического случая. Представлено успешное хирургическое лечение военнослужащего в военно-полевом госпитале с осколочным ранением шеи инородным металлическим телом (осколком снаряда), внутренней яремной вены, и флотирующим тромбом. Выполнена открытая операция — удаление инородного металлического тела и флотирующего тромба из внутренней яремной вены с ушиванием стенки вены полипропиленовой нитью 7/0. Послеоперационный период протекал без особенностей.
Выводы. В представленной работе продемонстрированы ранее не публиковавшиеся данные о варианте осколочного повреждения внутренней яремной вены. Предложенный способ хирургического лечения показал свою эффективность и безопасность.

Ключевые слова: тромбоз внутренней яремной вены; флотирующий тромб; осколочное ранение шеи; военно-полевая хирургия; инородное тело яремной вены; тромбэктомия

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Соответствие принципам этики: от пациента получено письменное информированное добровольное согласие на публикацию описания клинического случая, обезличенных медицинских данных и фотографий.

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INTRODUCTION

Military-field vascular surgery remains a relevant discipline within modern medicine of extreme situations. However, the existing literature lacks sufficient research dedicated to the surgical management of patients with vascular injuries sustained during combat operations. This trend can be traced back to the report by Oppel [1], published during World War I in 1915. At that time, military hospitals admitted rare patients with injuries to major arteries and veins due to high mortality from ongoing bleeding on the battlefield [1]. Consequently, the proportion of servicemen with such wounds did not exceed 1%, presenting as false aneurysms in 30% of cases and as hemorrhage in 70% [2].

According to Akhutin [3], until 1938, the most common surgical method for arterial and venous injuries in combat settings had been vessel ligation. Reconstructive surgeries became more widespread in military field hospitals during World War II. This shift was partially determined by the difficulty in evacuating casualties due to artillery shelling and the modernization of long-range weaponry. By April 1943, Petrovsky had performed 238 successful reconstructive procedures for major vascular injuries [4]. Among these, fragment wounds to the neck were of particular interest. The neck is the least protected body area, and bleeding from injuries to the brachiocephalic arteries and/or jugular veins cannot be controlled with a tourniquet on the battlefield, significantly reducing the wounded soldier's chances of evacuation to specialized medical care facilities.

The first documented experience in surgical management of neck injuries in servicemen was published by Pirogov [5] during the Eastern War of 1853–1856. He emphasized the importance of urgent surgical intervention in this cohort of patients [5]. During World War II, neck wounds accounted for 9.63–19.2% of all injuries, with fragment wounds comprising 68.4–74.0% of cases [6]. These figures highlight the high vulnerability of this anatomical region.

Surgical treatment of fragment wounds to major neck vessels remains critically relevant in modern combat operations. However, the available literature contains only sporadic reports focusing primarily on arterial injuries and surgical management of false aneurysms [7, 8]. This gap has resulted in the absence of a unified surgical approach for servicemen with injuries to the deep veins of the neck.

In this article, we report the outcome of surgical intervention in a patient with a fragment wound to the neck penetrating the left jugular vein and complicated by a floating thrombus.

CASE REPORT

A 28-year-old male serviceman sustained a fragment wound from an artillery shell explosion during a combat mission and was evacuated to a field hospital.

The patient's condition was assessed using military field surgery (MFS) scoring systems:

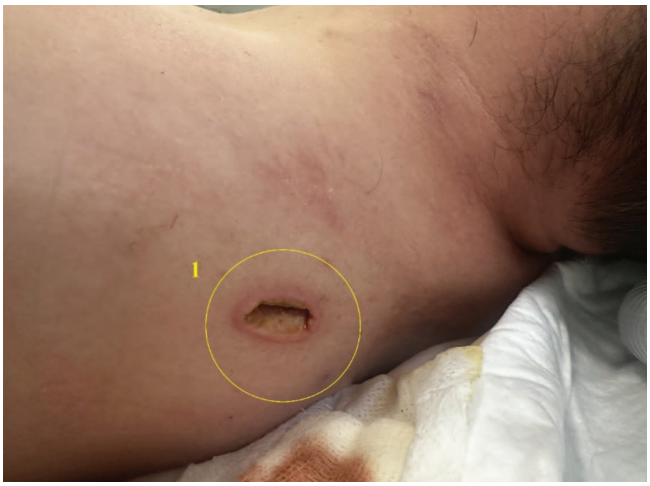
1. MFS-DS (Damage Severity Scale for MFS): 8.1 points — severe injury;
2. Abbreviated Injury Scale: 4 points — critical injury;
3. MFS-AS (Admission Severity Scale for MFS): 12 points — non-severe condition;
4. Revised Trauma Score: 7.8408 points — non-severe condition.

Consciousness was clear (15 points on the Glasgow Coma Scale). Complaints included pain in the left shoulder and left side of the neck.

Local findings: A 2 cm lacerated wound on the posterior surface of the left shoulder with scant serosanguineous discharge (Fig. 1).

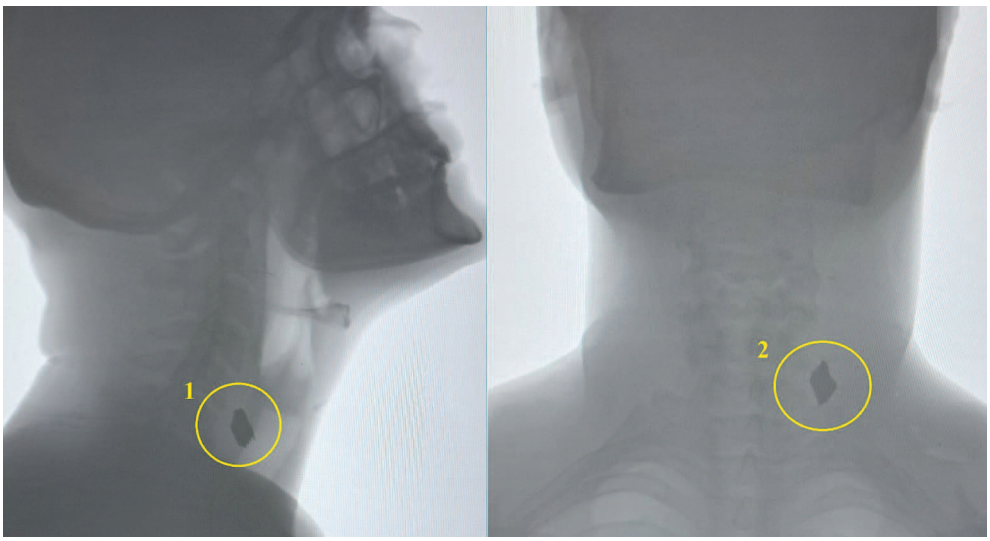
Neck radiography revealed a metallic foreign body (fragment) measuring 2x2 cm within the deep tissues of the left neck (Fig. 2).

According to ultrasonography (USG), the fragment was located between the left common carotid artery and the internal jugular vein (IJV), penetrating the IJV. A 3 cm floating thrombus attached to the fragment was visualized within the IJV lumen (Fig. 3).



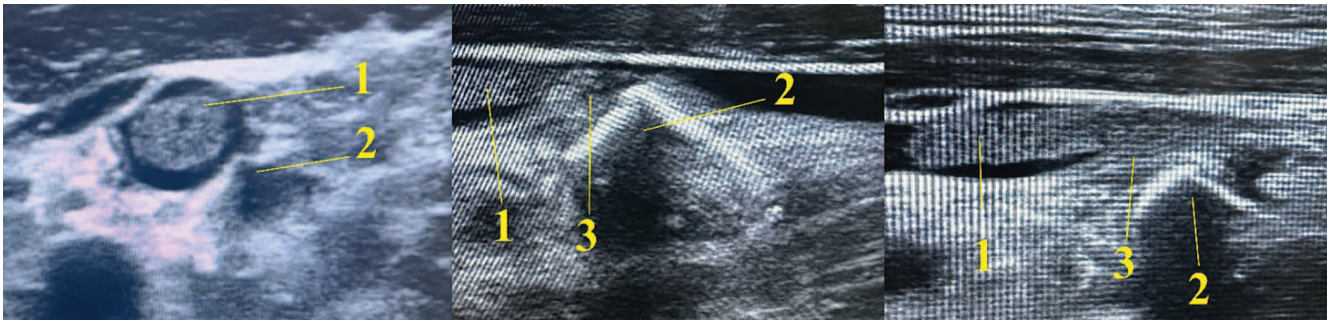
Photograph taken by the authors

Fig. 1. Fragment wound of the left shoulder: 1 — the entrance wound is located on the posterior surface of the left shoulder



Images obtained by the authors

Fig. 2. Neck radiography: 1 — lateral neck radiography: metallic foreign body (fragment) in the left neck tissues; 2 — anteroposterior neck radiography: metallic foreign body (fragment) in the left neck tissues



Images obtained by the authors

Fig. 3. Left neck ultrasound: 1 — floating thrombus in the lumen of the internal jugular vein; 2 — metallic foreign body (fragment) penetrating the lumen of the internal jugular vein; 3 — site of attachment of the floating thrombus to the metallic foreign body (fragment)

Diagnosis established: Combined fragment wound to the left neck and left upper extremity. A blind (non-penetrating) fragment wound to zone II of the left neck, resulting in injury to the internal jugular vein with formation of a floating thrombus. A blind fragment wound to the soft tissues of the left shoulder.

This injury is classified as severe according to the List of Injuries approved by Decree No. 855 of the Government of the Russian Federation (29.07.1998)[1].

The decision was made to perform surgical intervention comprising thrombectomy from the IJV with removal of the fragment (time from injury to surgery onset: 5 h).

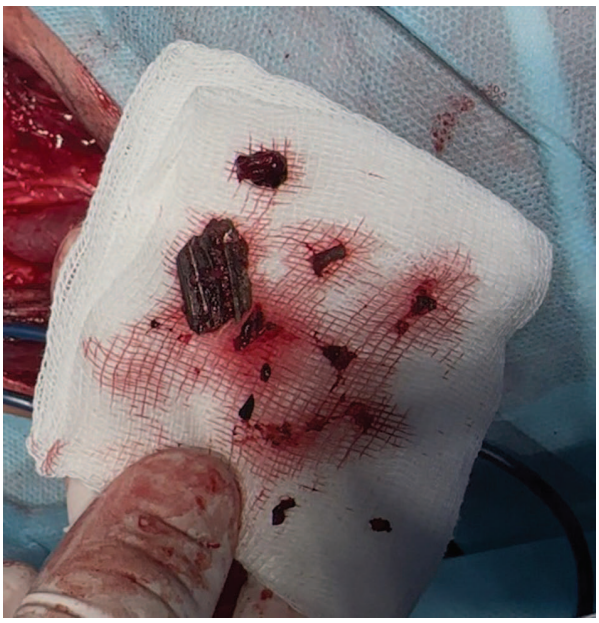
Procedure: A longitudinal incision was made along the lateral border of the left sternocleidomastoid muscle. The IJV was isolated. A 1 cm defect in the posterior wall of the IJV was identified, caused by foreign body invasion — a metallic fragment.

Heparin was administered intravenously in the amount of 5000 IU. The IJV was clamped 3 cm distal and proximal to the defect. A 2 cm venotomy of the anterior IJV wall was performed. The thrombus was removed from its lumen. Subsequently, the foreign body was extracted (Fig. 4).

The defect in the posterior wall of the IJV was repaired using a 7/0 polypropylene suture. Subsequently, a vascular suture of the venotomy site on the anterior wall was performed with 7/0 polypropylene. Clamps were removed, and satisfactory blood flow through the IJV was confirmed (Fig. 5).

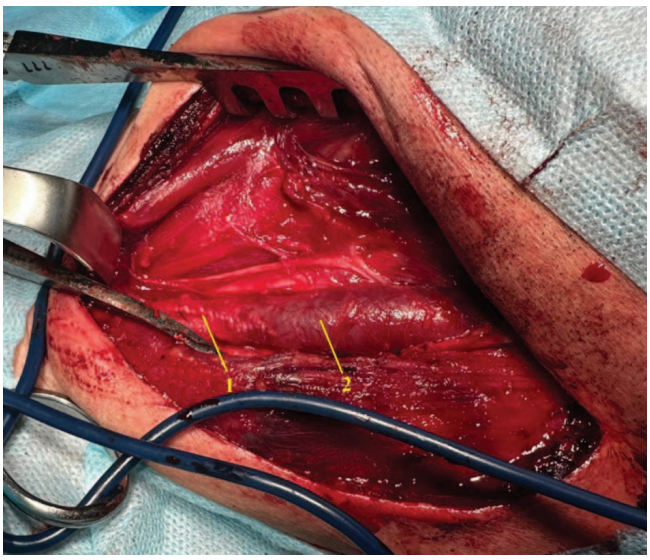
The postoperative course was uneventful. Sutures were removed on day 12 after surgery, after which the patient was discharged and evacuated to the next stage

[1] Decree No. 855 of the Government of the Russian Federation of 29.07.1998 “On Measures to Implement the Federal Law ‘On Compulsory State Insurance of Life and Health of Military Personnel, Citizens Called up for Military Training, Privates and Commanders of Internal Affairs Agencies of the Russian Federation, and Employees of Federal Tax Police Bodies’”.



Photograph taken by the authors

Fig. 4. Foreign metallic body (fragment) and thrombotic material removed from the internal jugular vein



Photograph taken by the authors

Fig. 5. Final result of the operation: 1 — closure of the venotomy site on the anterior wall of the internal jugular vein; 2 — internal jugular vein proximal to the site of reconstruction

of medical care. The following therapy was administered: apixaban 5 mg twice daily; ceftriaxone 1.0 g twice daily intramuscularly; analgesics.

According to ultrasonography findings, on the first day and on day 12 after surgery, the internal jugular vein (IJV) was patent, with no signs of thrombosis or restenosis.

DISCUSSION

The current literature lacks sufficient studies on the surgical management of neck injuries in servicemen during combat operations. Anipchenko et al. described a case of gunshot wound to the neck [9]. The fragment did not damage blood vessels, being localized paraesophageally in the Killian's triangle region. Subsequently, an abscess developed, requiring surgical intervention. Successful open surgery was performed to remove the foreign body and fragment. The postoperative course was uneventful [9]. This case highlights that foreign bodies (fragments) in deep neck tissues, even without causing bleeding, should be removed due to high risks of infectious complications, abscess formation, and mediastinitis.

Dadayan et al. presented a case of foreign body removal from the neck after fragment wound [10]. However, the fragment did not damage the neurovascular bundle. According to ultrasound, it was localized between the jugular vein and common carotid artery. The fragment was removed via open surgery under ultrasound guidance. The procedure was completed without complications [10]. The authors emphasized the importance of such operations due to risks of neurovascular bundle injury from foreign body migration.

Muminjonova et al. [7] reported a case of fragment wound to the neck with injury to carotid arteries, resulting in pseudoaneurysm formation. Aneurysm resection with subsequent prosthetic grafting was performed. This approach prevented potential aneurysm rupture-related bleeding and wound infection [7].

The clinical case presented in this article is the first report of a military-field surgical intervention describing surgical management of a neck fragment wound with fragment invasion into the IJV and floating thrombus formation. The technological complexity was due to the IJV wall defect being located on its posterior surface, preventing adequate thrombectomy and subsequent repair. Thus, it was decided to perform anterior IJV venotomy, open thrombectomy, followed by posterior wall repair and venotomy closure. A 7/0 polypropylene suture was used for vascular repair to minimize vein incorporation and prevent residual restenosis. In our opinion, IJV ligation without reconstruction could acutely impair cerebral venous outflow, leading to venous congestion and potential cerebral edema.

Thus, the implemented surgical strategy has proven successful and allowed optimal treatment outcomes to be achieved.

CONCLUSION

The presented case demonstrates previously unpublished data on a variant of fragment injury to the internal jugular vein. The proposed surgical technique has proven to be effective and safe. Its implementation prevented the development of distal embolism, wound infection, and bleeding.

References

1. Oppel VA. *Report of the Head of the Medical Unit of the Red Cross at the Caucasian Army, Professor V.A. Oppel.* Petrograd: Gov. print; 1915 (In Russ.).
2. Pchelina IV. Surgical care for the wounded with damage to the main vessels in wartime (on the 80th anniversary of the military operations at Lake Khasan). *Bulletin of the A.N. Bakulev Scientific Center for Cardiovascular Surgery, Russian Academy of Medical Sciences. Cardiovascular diseases.* 2018;19(S6):281 (In Russ.).
EDN: YNTCSL
3. Akhutin MN. *Surgical work during the battles at Lake Khasan.* Moscow: Medgiz; 1939 (In Russ.).
4. Petrovsky BV. *Surgical treatment of vascular injuries.* Moscow: Academy of Medical Sciences of the USSR' printing house; 1949 (In Russ.).
5. Pirogov NI. *Principles of General Military Field Surgery. Collected Works.* Volume 8. Moscow: Medgiz; 1961 (In Russ.).
6. Koroleva KYu, Savchenko IF, Borisov DN, Gaidakov GA. Medical and statistical characteristics of combat otolaryngological pathology. *Bulletin of the Russian Military Medical Academy.* 2020;39(S4):301–4 (In Russ.).
EDN: KMXQUS
7. Muminzhonova MM, Antonov GI, Chmutin GE, Miklashevich ER, Manukovskiy VA, Chmutin EG, et al. Gunshot wounds of the extracranial division of the carotid basin with the formation of false aneurism. Clinical observation staged treatment and literature review. *Bulletin of Neurology, Psychiatry and Neurosurgery.* 2025;4:480–92 (In Russ.).
https://doi.org/10.33920/med-01-2504-07
8. Kharchenko OYu, Kazantsev AN, Alekseev OV, Makhmudov RM, Kholmatov VN, Tenishev RR. Resection of false posttraumatic aneurysm of the axillary artery within a separate medical airmobile detachment in special military operation zone. *Pirogov Russian Journal of Surgery.* 2025;6:122–7 (In Russ.).
https://doi.org/10.17116/hirurgia2025061122
9. Anipchenko SN, Arkhangelsky DA, Kuznetsov SA, et al. Gunshot wound of the neck with a fragment localized in close proximity to the initial part of the esophagus with development of paraesophageal abscess. Surgical tactics. Surgical tactics. *Surgeon.* 2024;3–4:60–9 (In Russ.).
https://doi.org/10.33920/med-15-2402-06
10. Dadayan AR, Belik BM, Tenchurin RS, Bolotskov AS. Ultrasound-Guided Removal of Deep-Lying Foreign Bodies of the Soft Neck Tissue in a Patient with a Shrapnel Wound. *Journal of Experimental and Clinical Surgery.* 2024;17(2):66–71 (In Russ.).
https://doi.org/10.18499/2070-478X-2024-17-2-66-71

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MAYAK WORKER COHORT: CHARACTERISTICS AND KEY RESULTS OF EPIDEMIOLOGICAL STUDIES

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Introduction. The medical registry of workers at the Mayak Production Association (PA) was initially established with the purpose of studying the long-term stochastic health effects of occupational radiation exposure at the first nuclear industry enterprise in the USSR.
Objective. Assessment of radiogenic risk from prolonged occupational exposure among the Mayak PA worker cohort, including the subcohort of workers exposed to normal radiation conditions.
Materials and methods. This study represents one phase of a lifelong retrospective epidemiological investigation of health indicators, including the incidence and mortality from malignant neoplasms (MN), conducted within the framework of the medical-dosimetric registry of Mayak PA workers. The available study cohort is limited to employees of three main production facilities and two auxiliary plants, hired between 1948 and 1982. Within the study cohort, two subcohorts are distinguished based on factual data on radiation exposure levels and assessed medical outcomes. These include the subcohort of 1948–1958, personnel hired during the technology development phase and characterized by high occupational radiation exposure levels and that of 1959–1982, hired during routine operational periods with radiation doses comparable to modern limits. At the current stage, the attained age of workers in the second subcohort and the volume of accumulated data have enabled an analysis focused on individuals having worked under standard conditions, excluding the effects of high doses and dose rates. This has expanded the scope of statistically significant direct estimates of radiogenic MN risk. All studies of radiogenic risk in the cohort of Mayak PA workers were conducted using the Epicure statistical software package.
Results. The cohort comprised 25,755 workers. The vital status during the period of up to 31.12.2018 was known for 94% of subjects. In the 1948–1958 subcohort, the mean cumulative gamma radiation dose was 748 mGy, compared to 130 mGy in the 1959–1982 subcohort. Overall, 10,304 individuals (40.1% of the cohort) received low doses of gamma radiation. The mean cumulative lung dose from alpha radiation due to incorporated ^{239}Pu was 179.4 mGy, with 329.2 mGy and 41.0 mGy for the 1948–1958 and 1959–1982 subcohorts, respectively. The estimated excess relative risk per 1 Gy of alpha radiation lung dose was 3.5–8 for 60-year-old males. No deviations from linearity were found. Radiogenic risk decreased with an increase in age. A nonlinear dose-response relationship was identified for liver MN. The primary long-term effect of external gamma radiation was leukemia development, where a nonlinear model incorporating effect modification by age at exposure, time since exposure, and attained age provided better approximation than a linear model. For solid MN, the risk coefficient from external gamma radiation ranged 0.1–0.4 per 1 Gy. Among workers employed under normal radiation conditions (1959–1982 hiring period), the attributable risk assessment suggests that 1–5% of MN (excluding tumors in plutonium primary deposition organs) were radiation-induced, solely due to external gamma exposure.
Conclusions. The Mayak PA worker cohort, with its high-quality medical and dosimetric data, serves as a crucial source for direct epidemiological assessments of radiogenic risks from prolonged occupational radiation exposure. The identification of the routine production operation period not only validates the magnitude of carcinogenic risk but also highlights the need to extend both the follow-up period and the cohort itself to include more workers exposed to conditions comparable to modern standards.

Keywords: personnel; exposure; radiogenic risk; malignant neoplasms; non-tumor diseases; regulation; radiation safety

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Compliance with ethical principles: no bioethics committee approval was required as the study was based on archival data.

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КОГОРТА РАБОТНИКОВ ПО «МАЯК»: ХАРАКТЕРИСТИКА И ОСНОВНЫЕ РЕЗУЛЬТАТЫ ЭПИДЕМИОЛОГИЧЕСКИХ ИССЛЕДОВАНИЙ

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Введение. Регистр персонала ПО «Маяк» создан для исследования отдаленных стохастических медицинских последствий профессионального радиационного облучения работников первого в СССР предприятия ядерной промышленности.

Цель. Оценка радиогенного риска при пролонгированном профессиональном облучении в когорте работников ПО «Маяк», в том числе в когорте лиц, работавших в условиях штатной радиационной обстановки.

Материалы и методы. Выполненная работа является одним из этапов пожизненного ретроспективного эпидемиологического исследования показателей здоровья, в том числе заболеваемости и смертности от злокачественных новообразований (ЗНО), проводимого на базе медико-дозиметрического регистра работников ПО «Маяк». Доступная для исследованная когорта ограничена работниками трех основных производств и двух вспомогательных заводов, а также периодом найма на работу 1948–1982 гг. В исследуемой когорте, основываясь на фактических данных об уровнях облучения и полученных оценках медицинских последствий, выделены две субкогорты: 1948–1958 гг. — субкогорта найма в период освоения технологии и высоких уровней профессионального облучения и 1959–1982 гг. — субкогорта найма в период штатной эксплуатации производства и сопоставимых с современными пределами доз. На современном этапе достигнутый возраст работников, включенных во вторую субкогорту, и объем накопленных данных позволил провести анализ для лиц, работавших в штатных условиях, исключив влияние высоких доз и мощностей доз, и расширить область полученных статистически значимых прямых оценок радиогенного риска ЗНО. Все исследования радиогенного риска в когорте работников ПО «Маяк» проведены с использованием пакета для статистической обработки данных Epicure.

Результаты. Когорта состоит из 25 755 работников. Жизненный статус в период до 31.12.2018 известен для 94%. В субкогорте 1948–1958 гг. найма средняя накопленная доза гамма-облучения составила 748 мГр, 1959–1982 гг. — 130 мГр. В целом область малых доз гамма-излучения включала 10 304 (40,1% членов когорты) человека. Средняя накопленная доза в легких за счет альфа-облучения инкорпорированным ^{239}Pu составляла 179,4 мГр, для субкогорт 1948–1958 и 1959–1982 гг. — 329,2 и 41,0 мГр соответственно. Оценка избыточного относительного радиационного риска на 1 Гр дозы альфа-излучения в легких составила 3,5–8,0 на 1 Гр для мужчин в возрасте 60 лет. Не найдено отклонений от линейности. Радиогенный риск снижался с увеличением возраста. Выявлена нелинейная зависимость риска ЗНО печени. Основным отдаленным эффектом внешнего гамма-облучения являлось развитие лейкоза, для которого нелинейная зависимость с модификацией радиационного риска по временным характеристикам, связанным с возрастом на момент облучения, временем, прошедшим с момента облучения, и достигнутым возрастом является лучшей аппроксимацией, чем линейная. Для солидных ЗНО коэффициент риска от внешнего гамма-излучения составил 0,1–0,4 на 1 Гр. Среди лиц, работавших в условиях штатной радиационной обстановки (1959–1982 гг. найма), оценка атрибутивного риска ЗНО, за исключением опухолей органов основного депонирования плутония, позволяет отнести 1–5% случаев к радиационно-индуцированным, причем только вследствие влияния внешнего гамма-излучения.

Выводы. Когорта работников ПО «Маяк», обеспеченная высококачественными медико-дозиметрическими данными, является важным источником прямых эпидемиологических оценок радиогенного риска при профессиональном пролонгированном радиационном воздействии. Выделение периода штатной эксплуатации производства, с одной стороны, подтверждает величину канцерогенного риска, с другой — указывает на необходимость расширения периода наблюдения и самой когорты лиц, работавших в условиях, сопоставимых с современными.

Ключевые слова: персонал; облучение; радиогенный риск; злокачественные новообразования; неопухолевые заболевания; нормирование; радиационная безопасность

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Соответствие принципам этики: одобрение биоэтического комитета не требовалось, поскольку исследование выполнено на основе архивной информации.

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INTRODUCTION

Hygienic regulation of ionizing radiation is based on understanding its medical consequences. For this reason, since the first years of practical use of ionizing radiation, permissible exposure levels have decreased by more than an order of magnitude: from 500 mSv per year in the 1930s to 20 mSv per year today[1]. The primary reason for this gradual reduction in dose limits is related to the stochastic (carcinogenic) nature of the main adverse effects of ionizing radiation, which typically develop following long latency periods. To assess the risks associated with these effects, prolonged (and still ongoing) observation of irradiated populations is required—currently spanning a maximum of 70–75 years. During this period, methods for radiation-epidemiological studies have been developed, and estimates of radiogenic risk have been

[1] Romanovich IK, Balonov MI, Barkovsky AN, Brook GYa, Vishnyakova NM, Golikov VYu, et al. Comments on the Radiation Safety Standards (RSS-99/2009). Edited by Academician of the Russian Academy of Medical Sciences Onishchenko GG. St. Petersburg: Professor P.V. Ramzaev St. Petersburg Research Institute of Radiation Hygiene; 2012. EDN: YKYHSP

obtained (through epidemiological and radiobiological research)[2].

The selection, quality assessment, and evaluation of scientific research results are conducted by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Based on continuously updated data on the relationship between cancer incidence, mortality, and ionizing radiation doses, UNSCEAR systematically publishes scientific reports on the levels and consequences of radiation exposure to human health and the environment. These reports are recognized as a reliable and comprehensive source of information by the international community and are widely used for risk assessment and radiation protection measures. Radiation safety recommendations are formulated by the International Commission on Radiological Protection (ICRP). In the USSR and later in the Russian Federation, occupational dose limits for radiation workers have always aligned with ICRP guidelines [1].

The Life Span Study (LSS) of atomic bomb survivors in Hiroshima and Nagasaki (Japan) remains the primary source of quantitative radiogenic risk estimates, due to its large cohort size (over 100,000 subjects) and wide range of radiation doses (up to 4 Gy) [2]. The cohort includes both males and females of various ages at exposure (from children to the elderly), enabling robust population risk assessments[3]. In its latest Publication 103, providing recommendations for the radiological protection of workers and the public, the ICRP states:

"Risk modeling was based on data from the LSS cohort of Japanese atomic bomb survivors, but epidemiological literature was also reviewed to compare other studies with LSS-derived estimates."[4]

Thus, from a radiation safety perspective, the scientific community requires validation of LSS findings using data on the effects of occupational exposure among workers in radiation-hazardous industries.

The Mayak Production Association (PA) was the first nuclear industry enterprise in the USSR. The Mayak PA Personnel Registry was created as part of the Epidemiology Department to study long-term stochastic effects of occupational ionizing radiation exposure. Data collection began in the mid-1980s and continues up to the present [3].

The cohort derived from this registry differs from other similar cohorts [4–6], remaining the only global cohort demonstrating statistically significant effects from both alpha radiation (via incorporated plutonium) and external gamma exposure[5].

In this research, we aim to assess radiogenic risks from prolonged occupational radiation exposure in the Mayak PA worker cohort, including the subcohort employed under normal radiation conditions.

MATERIALS AND METHODS

Inclusion criteria for the study cohort and subgroup stratification

A long-term retrospective epidemiological study on the incidence and mortality from malignant neoplasms (MN) was conducted using the medical-dosimetric registry of Mayak Production Association (PA) workers. Initially, the Mayak PA registry contained information exclusively on personnel working during the 1948–1972 period at three main production facilities (reactors, radiochemical and chemical-metallurgical plants) [7]. Subsequently, the registry was extended to include data on workers hired during the following decade [8], as well as those from two auxiliary facilities, i.e., the water treatment plant and the mechanical repair plant. The registry continues to be updated both by adding newly hired workers at these facilities, currently including individuals employed up to 2016 [3], and by collecting data on employees from other departments. As of today, the Mayak PA medical-dosimetric registry covers the data on workers employed at the main plants and other enterprise divisions in 1948–2016.

The Mayak worker cohort, which is currently available for study, is limited to workers from three main and two auxiliary production facilities hired in 1948–1982. This restriction is related to insufficient and lower-quality dosimetric monitoring of personnel from other Mayak PA departments, particularly regarding internal exposure from incorporated radionuclides.

At the time of commissioning the Mayak PA, knowledge about the effects of radiation on the human body was limited. The delayed manifestation of health consequences also contributed to a lag in implementing more stringent radiation exposure limits. In the USSR, radiation safety standards were based on ICRP recommendations. The authors in [9] provide detailed information on the evolution of dose limits for radiation workers — from initial levels of 0.1 R/day and 30 R/year to the annual limit of 50 mSv recommended by the ICRP[6] and implemented through Regulation No. 333-60[7].

The Mayak PA personnel registry initially identified four subcohorts based on the year of employment at the main production facilities: 1948–1953, 1954–1958, 1959–1963, and 1964–1972 [10, 11]. Subsequently, the fifth subcohort (1973–1982) and workers from two auxiliary facilities were added [8]. Currently, based on actual radiation exposure levels and assessed health outcomes, two subcohorts have been distinguished:

- the 1948–1958 subcohort includes workers hired during the technology development phase with high occupational radiation exposures;

[2] ICRP Publication 103. Recommendations of the ICRP. Annals of the ICRP; 2008. https://doi.org/10.1016/j.icrp.2007.10.003
[3] ICRP Publication 26. ICRP. Recommendations of the ICRP. Ann. ICRP; 1977.
[4] ICRP Publication 103. Recommendations of the ICRP. Ann. ICRP; 2008. https://doi.org/10.1016/j.icrp.2007.10.003
[5] ICRP Publication 150. Cancer risk from exposure to plutonium and uranium. Ann. ICRP; 2021. https://doi.org/10.1177/01466453211028020
[6] ICRP. Publication 1. Recommendations of the International Commission on Radiological Protection. Pergamon Press, Oxford; 1977.
[7] Sanitary Regulations for Work with Radioactive Substances and Sources of Ionizing Radiation No. 333-60, approved by the Chief State Sanitary Physician of the USSR on 25.06.1960.

- the 1959–1982 subcohort includes workers hired during routine operations with exposure levels comparable to modern dose limits [8, 12].

All radiogenic risk studies in the Mayak worker cohort have employed methodologies and software tools, particularly the Epicure[8] statistical software package [13], consistent with those used in both the LSS cohort and other radiation worker cohorts worldwide. Tabulated data are presented with quantitative characteristics including median (M_e), minimum (min), and maximum (max) values.

RESULTS AND DISCUSSION

Cohort size and follow-up period

Table 1 presents the cohort and subcohort sizes along with the distribution of workers by sex, birth year, age at hiring, and employment duration. The cohort comprised 25,755 workers, including 25% females, with a wide range of birth years (1886–1965) and ages at employment initiation (18–69 years). The 1948–1958 hiring subcohort included 13,790 workers (53.5%), while the 1959–1982 subcohort contained 11,966 (46.5%). Due to sufficient availability of male specialists, females constituted only 20.7% in the latter subcohort, compared to 28.2% in the early post-war years. Most workers had already completed their employment at the enterprise — by 2018, 98% of workers had been discharged, including 100% from the first subcohort.

Information on the vital status of cohort members (specifically the year of departure from the city, location, death data) was collected and prepared for use in epidemiological studies through 2018 inclusive (Table 2). The vital status is known for 24,146 individuals (93.8%). Among those with the known vital status, 17,810 persons (73.8%) had died, with 89.0% deceased in the first decade of hire subcohort and 57.1% in the 1959–1982 hire subcohort. The increase in deaths in recent years (2009–2018) was substantial (23.3% of total deaths over the 70-year observation period). Extending the observation period through 31.12.2018 allowed accumulation of over 1 million person-years of follow-up for analysis of radiogenic mortality risk.

Cause-of-death and cancer incidence data

Cause of death was coded according to two International Statistical Classifications of Diseases[9] and Related Health Problems, 9th and 10th revisions (ICD-9, ICD-10). Both codes are provided for each worker.

For all individuals who died in the city, information on the cause of death was obtained from medical sources or civil registry records. Due to the availability of medical information among those who died in the city, the proportion of unknown causes of death is 1.6% for the entire observation period and 2.7% for 2010–2018.

For individuals who left the city, obtaining information on the cause of death from official sources is currently virtually impossible. However, even before the adoption of the Federal Law “On Personal Data,”[10] this was a challenging task. As a result, among those who left and died before the 2000s, the number of individuals with an unknown cause of death was ≈7%, while later—on average, about 50% (Fig.). Over the past 20 years, the primary source of data on the cause of death has remained personal contact with relatives.

The structure of causes of death differed slightly depending on the hiring period. On average, 47.8% of deaths were due to cardiovascular diseases, 24.3% to malignant neoplasms (MN), and 13.1% to external causes (Table 3).

Unlike mortality data, which were obtained for all members of the study cohort regardless of their place of residence, information on diseases is currently available only for the period when individuals resided in Ozersk. All cases were coded according to ICD-9 and ICD-10. Additionally, the data included morphological diagnoses of MN in accordance with the International Classification of Diseases for Oncology (ICD-O)[11].

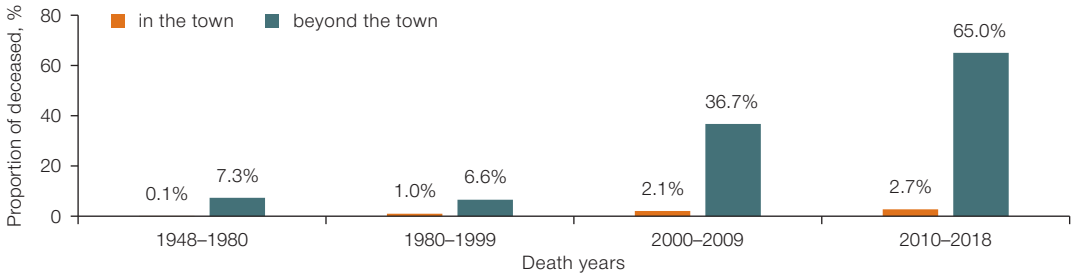

Figure prepared by the authors using data from the Mayak Production Association Personnel Registry

Fig. Proportion with unknown cause of death

[8] Preston DL, Lubin J, Pierce DA, McConney ME, Shilnikova NS. Epicure Manuals.URL: https://hirosoft.com/wp-content/uploads/nethelp/NetHelp/index.html#!Documents/userguide.htm (access date: 06.05.2025).
[9] International Classification of Diseases and Related Health Problems) ICD-10 Version:2019
[10] Federal Law No. 152-FZ of 27.07.2006 «On Personal Data».
[11] International Classification of Diseases for Oncology (ICD-O), 3rd ed., 1st revision. St. Petersburg: «Problems in oncology», 2017.

Table 1. Quantitative composition of the Mayak PA worker cohort

Numerical Profile	Hiring period		
	1948–1958	1959–1982	1948–1982
Number of workers, *n*	13 790	11 965	25 755
males	9907	9486	19 393
females	3883 (28.2%)	2479 (20.1%)	6362
Birth cohort			
before 1930	8080	1004	9084
1930–1950	5710	6867	12577
1950–1965	–	4094	4094
birth year range M_e (min–max)	1928 (1886–1942)	1944 (1893–1965)	1935 (1886–1965)
Age at hiring at Mayak PA, years			
<20	4369	5462	9831
20–30	7163	4372	11535
30–55	2243	2107	4350
55>	15	24	39
Age range M_e (min–max)	22.4 (14–65)	20.8 (14–69)	21.8 (14–69)
Duration of employment at Mayak PA, years			
<5	3624	2730	6354
5–20	5121	3571	8692
20–40	4001	4144	8145
40<	1044	1520	2564
employment duration (M_e)	11	18	14
Employment status			
dismissed	13 790	11 511	25 301
continue to work as of 2018	0	454	454

Table compiled by the authors using data from the Mayak PA Personnel Registry

A total of 4285 malignant neoplasm cases were diagnosed among 3805 workers in 1948–2018. Over the last 19 years of observation (2000–2018), the number of MN cases accounted for 49.2% (2107 cases)—nearly the same as during the previous 52 years (1948–1999; 2178 cases).

Dosimetric data

Dosimetric information represents fundamental data for epidemiological studies of radiogenic risk. Therefore, alongside cohort member identification, continuous updates of individual vital status data, and records of diagnosed diseases, in the 1990s, research began to revise and reconstruct absorbed doses (hereinafter referred to as doses) in specific organs from both external and internal exposure. As a result, five generations of dosimetric systems for external exposure dose assessments were sequentially developed (Doses-1999, Doses-2000, Doses-2005, Doses-2008, and Doses-2013), as well as seven generations for assessments of ^{239}Pu body content and corresponding internal exposure doses (Doses-1999, Doses-2000, Doses-2005, Doses-2008, Doses-2013, Doses-2016, and Doses-2019) [14–21].

Table 2. Vital status in the Mayak PA worker cohort (follow-up through 31.12.2018)

Numerical Profile	Hiring period						
	1948–1958		1959–1982		1948–1982		
	in the town	beyond the town	in the town	beyond the town	in the town	beyond the town	Total
Number of workers, *n*	6478	7311	8530	3436	15 008	10 747	25 755
traceable individuals:	6478	6148	8530	2990	15 008	9138	24 146
alive	693	696	3915	1032	4608	1728	6336
died	5785	5452	4615	1958	10 400	7410	17 810
lost to follow-up (abroad) (abroad)	0	1163 (131)	0	446 (146)	0	1609 (277)	1609
M_e of survival age, years	72.0	69.7	65.0	64.1	67.5	67.6	67.6
M_e of follow-up duration, years	45.8	44.8	40.9	41.5	42.3	43.4	42.5
Person-years of follow-up	285 621	298 546	338 662	134 088	624 283	432 634	1 056 917
Person-years of urban residence	348 938		369 600		718 538		

Table compiled by the authors using data from the Mayak PA Personnel Registry

Since the launch of the first industrial reactor at Mayak PA in 1948, the enterprise personnel have been provided with individual dosimeters for measuring gamma radiation doses [14–16]. Starting from 1984, systematic measurements of the neutron dose component have been introduced. Among the study cohort members, dosimetric data on external exposure is available for all 25,755 workers (100%), with 80% of annual dose estimates based on individual dosimeter readings. About 29% of cohort members have at least one annual dose estimated using only indirect data. For 2063 workers (8.0%), the analysis of professional employment records confirmed the absence of occupational external exposure.

The sets of annual external exposure dose values in different generations of dosimetric systems differ primarily in the list of organs for which doses were assessed and the size of the cohort. In 1949–1958, the average annual gamma radiation doses for personnel (Doses-2013, individual dose equivalent — γHp10) exceeded 50 mSv, decreasing to 5–10 mSv in 1968–1989. Since 1990, the average annual dose has not exceeded 5 mSv. Overall, 10,304 individuals (40.1% of the cohort) received low doses. The mean cumulative gamma dose was 748 mGy for the 1948–1958 hire subcohort and 130 mGy for the 1959–1982 subcohort.

Annual gamma doses were estimated through 2007. Due to the cessation of participation of Mayak PA specialists in joint studies, access to external dose data from 2008 onward has been restricted.

An analysis of autopsy materials from cohort workers revealed that internal exposure in the Mayak PA cohort essentially involved dosimetry of inhaled ^{239}Pu, compared to doses from uranium fission products, which were orders of magnitude lower [17, 18]. Estimates of nuclide content and organ/tissue doses are based on urinary ^{239}Pu activity measurements [19–21]. The latest Dose-2019 system includes dose estimates for 17 organs/tissues and lung compartments for 8395 workers. Cumulative doses varied significantly between primary plutonium deposition organs and systemic pool organs, with maximum values in bone surfaces and minimum values in stomach, intestines, and muscles.

The mean cumulative lung dose was 179.4 mGy (329.2 mGy for the 1948–1958 hiring period; 41.0 mGy for the 1959–1982 hiring period). In the first subcohort, 1394 workers (34.6%) received >100 mGy lung doses, compared to only 9.2% in the second subcohort. Conversely, 264 workers (6.5%) hired before 1959 and 1734 (39.7%) hired later received <5 mGy lung doses. Systemic organ doses were two orders lower: the mean stomach dose was 1.2 mGy, with >5 mGy doses found in 4.7% of examined workers (only 13 in the later subcohort).

Only 32.6% of workers in the study cohort underwent examination. As of 2018, approximately 2000 local residents remained available for testing, including < 200 early hires (first decade). For unexamined workers, doses were estimated using the Job Exposure Matrix (JEM) approach, covering 25,423 workers (98.7%).

The Mayak PA worker cohort remains the world's primary source on health effects of occupational plutonium exposure. The main stochastic effect of inhaled plutonium compounds is lung cancer. Numerous Mayak

Table 3. Structure of causes of death and malignant neoplasm incidence among Mayak PA workers (follow-up through 31.12.2018)

Cause of death / disease	Mortality, %	MN Incidence◆, %
Cause of death is known	15 767–100	–
Malignant neoplasms*	3837–24.3	4285–100
solid MN▲	3615–94.2	4056–94.7
stomach cancer∴	563–15.6	455–11.2
MN of colon, rectosigmoid junction and rectum	425–11.8	529–13.0
cancer of liver and intrahepatic bile ducts ∴	114–3.2	76–1.9
pancreatic cancer ∴	179–5.0	148–3.6
lung cancer ∴	1021–28.2	720–17.8
non-melanoma skin cancer ∴	18–0.5	571–14.1
breast cancer ▫	130–15.0	180–15.3
MN of female genital organs ▫	101–11.7	157–13.4
prostate cancer ▪	147–4.9	266–8.6
bladder cancer ∴	83–2.3	268–6.6
cancer of the kidneys, other and unspecified urinary organs ∴	105–2.9	161–4
unknown primary tumor ∴	151–4.2	40–1
hemoblastoses ▲	222–5.8	229–5.3
leukemias ∴	129–58.1	114–49.8
Diseases of the blood and blood-forming organs*	20–0.1	–
Diseases of the circulatory system*	7538–47.8	–
ischemic heart disease#	4067–54.0	–
cerebrovascular diseases#	2510–33.3	–
External causes*	2061–13.1	–
Other causes*	2311–14.7	–

Table compiled by the authors using data from the Mayak PA Personnel Registry

Note: * — % of known causes of death; ▲ — % of malignant neoplasms (NM); ∴ — % of solid NM; # — % of circulatory system diseases; ▫ — % of NM in women; ▪ — % of NM in men; ◆ — incidence data reflect diagnoses made exclusively within the Ozersk population; "–" — cases of benign or non-neoplastic nature fall outside the scope of this registry.

studies employing various dosimetric systems, observation periods, and non-radiation factors have established lung cancer dose-response models and statistically significant risk estimates [22–24].

The estimated excess relative radiation risk (ERR) per 1 Gy dose to the lungs was 3.5–8 per 1 Gy for males aged 60 years. No deviations from linear dose-response relationships were found. Radiogenic risk values showed a stronger dependence on smoking status than on gender, although these factors demonstrated moderate correlation (r = 0.61) in the Mayak PA worker cohort. Additionally, the excess risk showed a statistically significant decline with an increase in age.

Studies of the Mayak PA cohort also revealed dose-dependent relationships between alpha radiation dose and MNs in other primary plutonium deposition organs (liver, bones). For liver cancer, a nonlinear dose response was observed, although apparently being driven exclusively by high-dose exposures.

For other solid tumors as well as lymphohematopoietic malignancies, neither incidence nor mortality outcomes showed demonstrable effects from incorporated plutonium exposure levels.

Beyond plutonium-related effects, the Mayak worker cohort has provided estimates of radiogenic cancer risks from external gamma exposure. The principal late effect of gamma radiation in this cohort was leukemia development. The radiation risk for leukemia (excluding chronic lymphocytic leukemia) was approximately 3 per 1 Gy dose to red bone marrow under a linear model [25–27]. However, the data were statistically significantly better described by nonlinear (purely quadratic or linear-quadratic) models incorporating effect modification by:

- age at exposure,
- time since exposure,
- attained age [26, 27].

For solid MN, the coefficient of excess relative risk per Gy (ERR/Gy) from external gamma radiation ranged 0.1–0.4 per Gy across various studies [28–30]. When examining the influence of non-radiation factors (sex, smoking, type of production, attained age, age at hire) as modifiers of radiogenic risk, no statistically significant differences were found.

When developing models to predict MN risk among workers at modern facilities, it is important to consider the significant difference between the current working conditions, including dose loads, and those during the formative period [31–40]. The assessment of radiogenic risk for solid MN incidence (excluding MN in primary plutonium deposition organs) in relation to combined occupational gamma and alpha radiation levels among workers employed under normal radiation conditions (1959–1982 hiring period) revealed an increase in MN incidence at external radiation doses of 0.5–1.0 Gy (relative risk RR = 0.15; 95% CI: -0.21–0.51) and at alpha radiation doses up to 0.005 Gy (RR = 0.30; 95% CI: 0.07–0.53). The linear coefficient of radiation risk for MN incidence (ERR/Gy) depending on gamma radiation dose was statistically significantly different from zero only at the 90% level (0.36; 95% CI: -0.02–0.85; 90% CI: 0.03–0.76) when alpha radiation dose was not accounted for [41]. Estimates of the linear ERR/Gy coefficient for alpha radiation dose were negative[12].

In the study of cancer mortality using a linear dose-response function, the excess risk coefficient was zero for alpha radiation dose and positive, although not statistically significant, for gamma radiation dose (ERR: 0.17/Gy; 95% CI: -0.24–0.68)[13]. When conducting an interval dose estimation, a positive and statistically significant excess risk was observed only in the high-dose range of external radiation above 0.5 Gy (ERR: 0.33/Gy; 95% CI: 0–0.82). When modeling only alpha dose intervals, a statistically significant positive association was found in the dose range up to 0.005 Gy; however, this excess risk was not confirmed when using a model accounting for both radiation types [42].

Thus, among workers employed under normal radiation conditions (1959–1982 hiring period), the attributable risk assessment for MNs (excluding tumors in primary plutonium deposition organs) suggests that only 1–5% of cases can be considered radiation-induced, and solely due to external gamma radiation exposure.

In the analysis of non-cancer mortality rates among workers hired in 1959–1982[14], a comparison of various excess relative risk models based on external radiation exposure levels, both with and without consideration of internal exposure levels, showed no increase in mortality with an increase in radiation exposure. Indeed, no disease category showed a positive estimate of the ERR/Gy coefficient when using a linear dose-response relationship, nor was there a monotonic statistically significant increase in relative risk when using a nonparametric dose-response relationship.

The improvement in data approximation quality when using dose intervals was statistically significant at the 90% level only for the group of infectious and parasitic diseases: however, this was solely due to a positive estimate of excess risk in the dose interval up to 100 mGy (ERR = 0.6; 90% CI: 0.04–1.58). For the most representative category of circulatory system diseases, no dose-effect relationship was observed as well, with the only positive estimate of excess risk obtained for doses exceeding 0.5 Gy (ERR = 0.05; $p > 0.5$).

CONCLUSION

The Mayak Production Association Personnel Registry constitutes an authoritative source for epidemiological assessments of radiogenic risks associated with prolonged occupational radiation exposure at nuclear industrial facilities. Based on the worker cohort hired in 1948–1982, direct estimates of carcinogenic risk have been obtained for both external radiation doses and ^{239}Pu intake. The observation of workers who began employment during 1959–1982 serves dual purposes. On the one hand, this allows the magnitude of dose-dependent carcinogenic risk from cumulative gamma radiation exposure to be assessed. On the other hand, this work highlights the need to extend both the observation period and the cohort itself to include personnel working under exposure conditions comparable to contemporary standards.

[12] Indicators and Risk Prognosis for Long-Term Medical Consequences of Prolonged Exposure to Ionizing Radiation from External and Incorporated Sources Among Personnel at the Nuclear Industry Enterprise 'Mayak' PA Under Normal Operating Conditions, and Assessment of Medical-Demographic Health Indicators of the Population Living Near the Radiation-Hazardous Facility. Research Report (Interim). Federal State Budgetary Scientific Institution 'South Urals Institute of Biophysics', Head: Sokolnikov ME. Ozersk: 2023. State Research Registration No. 122041300044-3. Deposited at CITIS 07.02.2025, No. IKRBS I224120300119-7 / 225020709083-0.

[13] Ibid.

[14] Ibid.

References

1. Panfilov AP. Evolution of the system supporting radiation safety in nuclear industry of Russia and its current status. *Radiation and Risk*. 2016;25(1):47–64 (In Russ.). EDN: VZDXED
2. Grant EJ, Brenner A, Sugiyama H, Sakata R, Sadakane A, Utada M, et al. Solid Cancer Incidence among the Life Span Study of Atomic Bomb Survivors: 1958–2009. *Radiation Research*. 2017;187(5):513–37. https://doi.org/10.1667/RR14492.1
3. Sokolnikov ME, Kabirova NR, Okatenko PV, Koshurnikova NA, Tsareva YuV, Martinenko IA, et al. Medical-dosimetry registry of the personnel of "Mayak production association": status and perspectives. *Journal of Radiation Sahety Issues*. 2023;3(111):42–55 (In Russ.). EDN: WWGSJD
4. Boice JD, Cohen SS, Mumma MT, Ellis ED. The Million Person Study, whence it came and why. *International Journal of Radiation Biology*. 2022;98(4):537–50. https://doi.org/10.1080/09553002.2019.1589015
5. Hunter N, Haylock RGE, Gillies M, Zhang W. Extended analysis of solid cancer incidence among the Nuclear Industry Workers in the UK: 1955–2011. *Radiation Research*. 2022;198(1):1–17. https://doi.org/10.1667/RADE-20-00269.1
6. Richardson DB, Leuraud K, Laurier D, Gillies M, Haylock R, Kelly-Reif K, et al. Cancer mortality after low dose exposure to ionising radiation in workers in France, the United Kingdom, and the United States (INWORKS): cohort study. *BMJ*. 2023;16(382):e074520. https://doi.org/10.1136/bmj-2022-074520
7. Koshurnikova NA, Shilnikova NS, Okatenko PV, Kreslov VV, Bolotnikova MG, Sokolnikov ME, et al. Characteristics of the cohort of workers at the Mayak nuclear complex. *Radiation Research*. 1999;152(4):352–63. https://doi.org/10.2307/3580220
8. Koshurnikova NA, Shilnikova NS, Sokolnikov ME, Bolotnikova MG, Okatenko PV, Vasilenko EK, et al. Medical-dosimetry registry of workers at the "Mayak" production association. *International Joural of Low Radiation*. 2006;2(3/4):236. https://doi.org/10.1504/IJLR.2006.009516
9. Onischenko GG, Romanovich IK, Istorik OA, Vodovatov AV, Biblin AM, Kormanovskaya TA, et al. On the 125th anniversary of the discovery of radioactivity: history of development and current state of regulation of the provision of the radiation safety of the public. *Radiation Hygiene*. 2021;14(4):6–16 (In Russ.). https://doi.org/10.21514/1998-426X-2021-14-4-6-16
10. Koshurnikova NA, Shilnikova NS, Okatenko PV, Kreslov VV, Bolotnikova MG, Sokolnikov ME, et al. Characteristics of the cohort of workers of the nuclear enterprise "Mayak PA" (Part I). *Radiation Safety Problems*. 1998;2:46–55 (In Russ.). EDN: WBJZRP
11. Koshurnikova NA, Shilnikova NS, Okatenko PV, Kreslov VV, Bolotnikova MG, Sokolnikov ME, et al. Characteristics of the cohort of workers of the nuclear enterprise "Mayak PA" (Part II). *Radiation Safety Problems*. 1998;3:48–58 (In Russ.). EDN: WBJZRP
12. Koshurnikova NA, Okatenko PV, Shilnikova NS, Kuznetsova IS, Sokolnikov ME. Health effects of occupational radiation exposure (cancer mortality among employees of the main plants of the Mayak production association). *Extreme Medicine*. 2006;2(16):5–14 (In Russ.). EDN: WLKKQZ
13. Nikipelov BV, Lyzlov AF, Koshurnikova NA. Experience of the first nuclear industry enterprise (levels of radiation and health of personnel). *Priroda*. 1990;2:30–8 (In Russ.). EDN: WBJMEB
14. Vasilenko EK. Dosimetry of external irradiation of manufacturers of PA Mayak: devices, methods, and monitoring results. In: Kiselev MF, Romanov SA, ed. *Sources and effects of irradiation of manufacturers of PA "Mayak" and the population located on the territory of the industrial enterprise*. Ozyorsk; 2009:66–135 (In Russ.). EDN: ZBOYBT
15. Napier BA. The Mayak worker dosimetry system (MWDS-2013): an introduction to the documentation. *Radiation Protection Dosimetry.* 2017;176(1–2):6–9. https://doi.org/10.1093/rpd/ncx020
16. Hohrjakov VF, Hohrjakov VV, Suslova KG, Vostrotin VV, Shhadilov AE, Sokolova AB, et al. Progress in plutonium dosimetry development at Mayak PA. *Radiation Safety Problems*. 2006;41(1):59–80 (In Russ.). EDN: JUURIP
17. Efimov AV, Sokolova AB. History and program of biophysical examinations in the epidemiological cohort of employees of the PA "Mayak". *Radiation and Risk*. 2024;33(4):131–43 (In Russ.). https://doi.org/10.21870/0131-3878-2024-33-4-131-143
18. Khokhryakov VV, Khokhryakov VF, Suslova KG, Vostrotin VV, Vvedensky VE, Sokolova AB, et al. Mayak Worker Dosimetry System 2008 (MWDS-2008): Assessment of Internal Dose from Measurement Results of Plutonium Activity in Urine. *Health Physics*. 2013;104(4):366–78. https://doi.org/10.1097/HP.0b013e31827dbf60
19. Birchall A, Vostrotin VV, Puncher M, Efimov AV, Dorrian M-D, Soolova AB, et al. The Mayak worker dosimetry system (MWDS-2013) for internally deposited plutonium: an overview. *Radiation Protection Dosimetry.* 2017;176(1–2):10–31. https://doi.org/10.1093/rpd/ncx014
20. Vostrotin VV, Napier BA, Zhdanov AV, Miller S, Sokolova AB, Bull RK, et al. The Mayak worker dosimetry system (MWDS-2016): internal dosimetry results and comparison with MWDS-2013. *Radiation Protection Dosimetry.* 2019;184(2):201–10. https://doi.org/10.1093/rpd/ncy200
21. Sokolnikov ME, Gilbert ES, Preston DL, Ron E, Shilnikova NS, Koshurnikova NA, et al. Lung, liver and bone cancer mortality in Mayak workers. *International Journal of Cancer.* 2008;123(4):905–11. https://doi.org/10.1002/ijc.23581
22. Gilbert ES, Sokolnikov ME, Preston DL, Schonfeld SJ, Schadilov AE, Vasilenko EK, et al. Lung Cancer Risks from Plutonium: An Updated Analysis of Data from the Mayak Worker Cohort. *Radiation Research.* 2013;179(3):332–42. https://doi.org/10.1667/RR3054.1
23. Labutina EV, Kuznetsova IS, Hunter N, Harrison J, Koshurnikova NA. Radiation Risk of Malignant Neoplasms in Organs of Main Deposition for Plutonium in the Cohort of Mayak Workers with Regard to Histological Types. *Health Physics*. 2013;105(2):165–76. https://doi.org/10.1097/HP.0b013e31828f57df

24. Shilnikova NS, Preston DL, Ron E, Gilbert ES, Vassilenko EK, Koshurnikova NA, et al. Cancer Mortality Risk among Workers at the Mayak Nuclear Complex. *Radiation Research*. 2003;159(6):787–98. https://doi.org/10.1667/0033-7587(2003)159
25. Kuznetsova IS, Labutina EV, Hunter N. Radiation Risks of Leukemia, Lymphoma and Multiple Myeloma Incidence in the Mayak Cohort: 1948–2004. *PLoS ONE*. 2016;11(9):e0162710. https://doi.org/10.1371/journal.pone.0162710
26. Sokolnikov ME, Preston DL, Stram D, Martinenko IA, Koshurnikova NA. Chronic effect of ionizing radiation and leukomogenic risk. *Chronic radiation exposure: late medical and biological effects.* Proceedings of the 7th scientific conference. Chelyabinsk; 2022 (In Russ.).
27. Sokolnikov M, Preston D, Stram DO. Mortality from solid cancers other than lung, liver, and bone in relation to external dose among plutonium and non-plutonium workers in the Mayak Worker Cohort. *Radiation and Environmental Biophysics*. 2017;56(1):121–5. https://doi.org/10.1007/s00411-016-0670-5
28. Sokolnikov ME, Preston DL, Gilbert E, Schonfeld S, Koshurnikova NA. Radiation Effects on Mortality from Solid Cancers Other than Lung, Liver, and Bone Cancer in the Mayak Worker Cohort: 1948–2008. *PLoS ONE*. 2015;10(2):e0117784. https://doi.org/10.1371/journal.pone.0117784
29. Hunter N, Kuznetsova IS, Labutina EV, Harrison JD. Solid cancer incidence other than lung, liver and bone in Mayak workers: 1948–2004. *British Journal of Cancer*. 2013;109(7):1989–96. https://doi.org/10.1038/bjc.2013.543
30. Wakeford R. What about the workers? *Journal of Radiology Protection.* 2024;44(2):011504. https://doi.org/10.1088/1361-6498/ad4eea
31. Laurent O, Samson E, Caer-Lorho S, Fournier L, Laurier D, Leuraud K. Updated mortality analysis of SELTINE, the French cohort of nuclear workers, 1968–2014. *Cancers.* 2022;15(1):79. https://doi.org/10.3390/cancers15010079
32. Haylock RGE, Gillies M, Hunter N, Zhang W, Phillipson M. Cancer mortality and incidence following external occupational radiation exposure: an update of the 3rd analysis of the UK National Registry for Radiation Workers. *British Journal of Cancer*. 2018;119(5):631–7. https://doi.org/10.1038/s41416-018-0184-9
33. Hunter N, Haylock R. Extended analysis of solid cancer incidence among nuclear industry workers in the UK 1955–2011: comparison of workers first hired in earlier and later periods. *Journal of Radiology Protection*. 2024;44(2):021515. https://doi.org/10.1088/1361-6498/ad4c72
34. Kelly-Reif K, Bertke SJ, Daniels RD, Richardson DB, Schubauer-Berigan MK. Ionizing radiation and solid cancer mortality among US nuclear facility workers. *International Journal of Epidemiology*. 2023;52(4):1015–24. https://doi.org/10.1093/ije/dyad075
35. Richardson DB, Daniels RD, Hamra GB, Leuraud K, Thierry-Chef I. Risk of cancer from occupational exposure to ionising radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS). *BMJ*. 2015;351:h535. https://doi.org/10.1136/bmj.h5359
36. Wakeford R. Overview of epidemiological studies of nuclear workers: opportunities, expectations, and limitations. *Journal of Radiological Protection*. 2021;41(4):1075. https://doi.org/10.1088/1361-6498/ac0df4
37. Cardis E, Vrijheid M, Blettner M, Gilbert E, Hakama M, Hill G, et al. The 15-country collaborative study of cancer risk among radiation workers in the nuclear industry: estimates of radiation-related cancer risks. *Radiation Research.* 2007;167(4):396–416. https://doi.org/10.1667/RR0553.1
38. Wakeford R. Solid cancer mortality among US radiation workers. *International Journal of Epidemiology.* 2023;52(6):1992–4. https://doi.org/10.1093/ije/dyad131
39. Schubauer-Berigan MK, Daniels RD, Bertke SJ, Tseng C-Y, Richardson DB. Cancer mortality through 2005 among a pooled cohort of U.S. nuclear workers exposed to external ionizing radiation. *Radiation Research*. 2015;183(6):620–31. https://doi.org/10.1667/RR13988.1
40. Kuznetsova IS. Comparison of Radiation Risk of Cancer Incidence among PA Mayak Workers Hired at Different Calendar Periods. *Medical Radiology and Radiation Safety*. 2021;66(6):50–6 (In Russ.). https://doi.org/10.12737/1024-6177-2021-66-6-50-56
41. Kuznetsova IS. Radiation risk of mortality from malignant neoplasms among workers hired at different periods of Mayak PA activity. *Radiation Safety Problems*. 2023;4(112):82–9 (In Russ.). EDN: ZMZQJW

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PROSPECTS FOR THE USE OF ALPHA-2-MACROGLOBULIN AS A RADIOPROTECTIVE AGENT

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Introduction. The diversity of clinical manifestations of radiation sickness creates significant difficulties in the development of a versatile means for the prevention and treatment of radiation injuries.
Objective. Assessment of the prospects for using alpha-2-macroglobulin (α2M) as a radioprotective agent.
Discussion. The existing agents were established to be incapable of simultaneous implementation of multiple mechanisms of radioprotective action, rendering the development of complex formulations the primary research direction. However, the toxicity, side effects, and multidirectional nature of many radioprotectors hinders their combined application. Along with inhibiting proteinases, alpha-2-macroglobulin (α2M) is involved in lipid metabolism and regulation of the antioxidant system. It influences enzyme activity, binds and transports numerous cytokines, affects the functions of immunocompetent cells, and controls the development of the inflammatory response and tissue remodeling processes. A number of published studies confirm α2M to be a promising radioprotector and a key component of innate radioprotection.
Conclusions. Preparations based on human blood polyfunctional proteins can serve as a basis for the development of means for preventing and treating radiation injuries. The α2M administration into the body reduces lethality, protects DNA from damage, lowers the oxidative stress level, mitigates the severity of leukopenia and thrombocytopenia, and reduces the number of necrosis foci. Further research into the radioprotective properties of this protein and the optimization of methods for its isolation from blood for industrial-scale production are required.

Keywords: alpha-2-macroglobulin; radioprotectors; radiation sickness; therapy of radiation injuries; radioprotective agents

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ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ АЛЬФА-2-МАКРОГЛОБУЛИНА КАК ПРОТИВОЛУЧЕВОГО СРЕДСТВА

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Введение. Многообразие клинических проявлений лучевой болезни создает значительные сложности в разработке универсального средства профилактики и терапии радиационных поражений.
Цель. Оценка перспектив использования альфа-2-макроглобулина (α2-МГ) как противолучевого средства.
Обсуждение. Установлено, что существующие средства не позволяют одновременно реализовать несколько механизмов противолучевого действия, основным направлением является разработка комплексных рецептур. Однако многие радиопротекторы токсичны и имеют побочные эффекты, разнонаправленность их воздействия препятствует комплексному применению. Известно, что α2-МГ, помимо ингибирования протеиназ, задействован в обмене липидов и регуляции антиоксидантной системы, влияет на активность ферментов, связывает и транспортирует многие цитокины, воздействует на функции иммунокомпетентных клеток, контролирует развитие воспалительной реакции и процессы ремоделирования тканей. Опубликован ряд работ, подтверждающих, что α2-МГ является перспективным радиопротектором и основным компонентом врожденной радиозащиты.
Выводы. Препараты полифункциональных белков крови человека могут служить основой для разработки средств профилактики и лечения радиационных поражений. Введение α2-МГ в организм снижает летальность, защищает ДНК от повреждения, снижает уровень окислительного стресса, уменьшает выраженность лейкопении и тромбоцитопении, количество очагов некроза. Требуются дополнительные исследования радиозащитных свойств данного белка и оптимизация методов выделения из крови под производственные нужды.

Ключевые слова: альфа-2-макроглобулин; радиопротекторы; лучевая болезнь; терапия радиационных поражений; противолучевые средства

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Финансирование: работа выполнена без спонсорской поддержки.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The current stage of technological development is associated with aggravation of radiation hazards to the level of public discussions around the possibility of using tactical nuclear weapons. At the same time, the delayed effects of radiation sickness are generally ignored, ranging from acute leukemia, which developed en masse 9–10 days after the bombings of Hiroshima and Nagasaki [1], to teratogenic effects observed in civilians living for many years in areas adjacent to territories contaminated with depleted uranium (Iraq, Serbia, Libya, Somalia, Haiti, etc.) [2]. It should be noted that the existing technologies aimed at ecosystem restoration and human safety, including biorevitalization and biomineralization methods, demonstrate insufficient efficiency [3]. Undoubtedly, the tragedy of Hiroshima and Nagasaki was a harsh lesson to learn for contemporaries, intensifying experimental studies of radiation sickness in laboratory animals, as well as the development of personal protective equipment and medical means of radiological protection.

Today, the biological effects of ionizing radiation are viewed as a combination of molecular, biochemical, morphological, physiological, and genetic changes. Numerous deterministic and stochastic (dose-independent) effects have been described and studied [4]. Overall, radiation pathology is characterized by a diversity of clinical forms, hindering the creation of a unified classification of radiation injuries. In addition to the duration of exposure and dose, the routes of radionuclide intake into the body, distribution features, tropism to organs and tissues, and the ability to adapt and regenerate at the individual level are of great importance for pathogenesis. With an increase in the absorbed dose, the bone marrow and intestines are sequentially affected, vascular-toxemic, cardiovascular, and cerebral forms of acute radiation sickness develop, with a corresponding reduction in the time to lethal outcome (a dose of up to 10 Gray is considered treatable) [4]. The diversity of clinical manifestations of radiation sickness creates significant barriers to the development of versatile prevention and treatment means.

The aim of this work is to assess the prospects for using alpha-2-macroglobulin as a radioprotective agent.

MATERIALS AND METHODS

The literature search was carried out in electronic bibliographic databases in the Russian (E-library, CyberLeninka) and English (PubMed) languages and patent sources (FIPS, EspaceNet). Search queries included the following keywords: alpha-2-macroglobulin, radioprotector, radioprotection, anti-radiation. The search depth for the combination of keywords “alpha-2-macroglobulin” and others was not specified, and the search depth for the remaining keywords was 10 years.

RESULTS AND DISCUSSION

Existing and novel radioprotective agents

Medicinal products used for the prevention and treatment of radiation injuries lack a unified classification; however, they can be conventionally divided into radioprotectors, agents for long-term enhancement of body resistance, and means for injury prevention. Based on the scenarios of exposure, they are distinguished as radioprotectors (providing short-term effects), radiomitigators (long-term exposure, stimulation of repair), radiomodifiers (non-specific enhancement of body resistance), agents preventing incorporation and promoting the elimination of radionuclides from the body, and means for suppressing undesirable body reactions to irradiation. Medical means are also conventionally subdivided into preventive (radioprotectors, stimulators of body radioresistance), therapeutic-prophylactic (radiomitigators, agents for alleviating the primary body reaction and means for preventing effects from incorporated nucleotides), and therapeutic agents (treatment of acute bone marrow syndrome, skin and mucous membrane injuries). Additionally, classifications based on the biological activity of the agents and other criteria exist [4, 5].

It is important to note that none of the hypotheses regarding the radioprotective action of pharmacological compounds allow for a unified theoretical generalization of the mechanism of their action, as none of the recommended or developed agents enable the simultaneous implementation of multiple mechanisms of radioprotective action [6]. In Russia, indralin (B-190) [4, 6], a biogenic amine, is recommended as a radioprotector. The high protective efficacy of indralin is primarily associated with its vasoconstrictor activity, leading to regional impairment of blood supply, including in radio-sensitive tissues. It has been suggested that B-190 provokes tissue hypoxia by activating tissue respiration through α1-adrenergic receptors. A radiomitigating effect has also been assumed; thus, indralin indirectly releases serotonin from bone marrow tissues, and serotonin, in turn, stimulates the proliferation of hematopoietic stem cells. However, the radiomitigating properties of indralin, while contributing to increased radioresistance, are still not comparable to its radioprotective effect [6].

Along with B-190, naphthyzin, a common alpha-adrenomimetic with vasoconstrictive action, is also used as a radioprotector. Recommended as a means for preventing and alleviating the primary radiation reaction, ondansetron hydrochloride dihydrate (Latran®, granisetron) is essentially an antiemetic drug [5, 6]. Enterosorbents and other means for preventing injuries from incorporated radionuclides, including potassium iodide, potassium-iron hexacyanoferrate (Ferrocyn®), calcium-trisodium salt of diethylenetriaminepentaacetic acid (Pentacin), and 2,3-dimercaptopropanesulfonate (Unithiol) [5, 6], protect only specific organs and have application limitations,

with many enterosorbents developed in the USSR long being discontinued.

The attempts to develop and test novel means of radiation protection are ongoing. Some previously developed anti-radiation agents (cystamine, Mexamine®) have been surpassed in terms of tolerability and protective properties by modern radioprotectors and are seeking new medical applications [6]. Efforts are being made to reduce the side effects of amifostine (WR-2721 is the primary radioprotector in the USA and Western Europe) by modifying the active component. Thus, a new polycysteine peptide with three thiol groups has been synthesized to reduce toxicity, demonstrating efficacy comparable to that of amifostine and a better safety profile [7].

Among original innovations, the proposal to use molecular hydrogen for radioprotection (as an antioxidant, anti-inflammatory, anti-apoptotic agent, and a factor influencing gene expression) can be mentioned. Protective effects on cognitive functions, the immune system, lungs, heart, digestive organs, hematopoietic organs, testes, skin, and cartilage tissues have been reported when administering hydrogen-enriched water to small laboratory animals. In patients undergoing radiation therapy for therapeutic purposes, the intake of such enriched water reduced side effects without affecting the primary treatment outcome. Inhalation of gaseous H_2 in terminal-stage cancer patients improved hematopoietic function [8].

Among substances of plant origin, celastrol (tripterin) has been described. This is a pentacyclic triterpenoid from the quinone methide family, derived from the roots of Chinese medicinal plants (*Tripterygium wilfordii* or *Celastrus regelii*). According to the authors, this substance is capable of inhibiting NF-κB pathways, exhibiting antioxidant activity, suppressing lipid peroxidation and oxidative DNA damage, and increasing animal survival in experiments [9].

As a separate research direction, the administration of bacterial strains to enhance survival has been described. In particular, data were published showing that the introduction of a radioresistant variant of *St. aureus* followed by irradiation of animals at the LD100/30 level increased survival by 77.7% [10]. It is assumed that the modified strains actively synthesize antioxidant factors affecting various organs and systems, which collectively enhance the body's resistance.

Since the creation of an original low-molecular-weight chemical compound with multi-targeted actions and diverse biological activities presents a significant challenge, research groups have long adopted the strategy of utilizing natural creations. In this regard, cytokines, hormones, and vitamins have been studied as a means for the prevention and early therapy of radiation injuries [5]. Over a decade ago, the U.S. Food and Drug Administration (US FDA) approved a number of radioprotective agents (radiomitigators), including 5-androstenediol (neumune), genistein (BIO 300), protein kinase ON01210 (Ex-RAD), Toll-like receptor 5 agonist CBLB502 (entolimod), the corticosteroid beclomethasone (OrbeShield®), recombinant human interleukin-12 (HemaMax®), and recombinant growth factor G-CSF (Neupogen®) [5]. An analogue of the latter agent (filgrastim) is also positioned as a means for the pathogenetic therapy of acute radiation sickness [6], although it is essentially a genetically engineered granulocyte colony-stimulating factor that stimulates leukopoiesis. Beta-leukin, which is no longer produced but was recommended as an effective radiomitigator for early use after accidental irradiation [5, 6], is a recombinant analogue of human pro-inflammatory interleukin-1β. While the aforementioned natural compounds and their recombinant analogues demonstrate certain levels of radioprotective activity — either directly or indirectly (e.g., by stimulating an inflammatory response that counteracts processes developing during radiation sickness) — and protect specific organs and systems from the progression of radiation sickness, they still fail to provide a comprehensive protection for the entire organism.

According to a number of radiology specialists, the primary direction in the development of approaches to pharmacological correction of the initial response to irradiation is the creation of complex formulations. Their components should effectively target various organs and systems, and accordingly, different links in the pathogenesis of early disorders [5]. A consequence of this approach has been a striking diversity of means for preventing and treating individual manifestations of radiation sickness, albeit with questionable acceptability of the results from their combined use.

This diversity is exemplified by Chinese authors [11], who illustrated all radioprotective means mentioned in scientific publications (Fig. 1). Among the listed agents, along with "classical" amifostine, are the non-steroidal anti-inflammatory benzydamine for treating oral mucositis, glutamine for mucosal restoration, pentoxifylline with statins to reduce inflammation and prevent fibrosis, meloxicam as an anti-proliferative agent, and superoxide dismutase for protection against free radical damage. The authors also mentioned Toll-like receptor agonists, low-molecular nitroxide compounds, and sphingosine-1-phosphate.

The natural radioprotective agents described so far include plant extracts (flavonoids, etc.), vitamins (A, C, E), trace elements (selenium), and components of bacterial lysates (flagellin). Cytokines (including a number of pro-inflammatory cytokines and growth factors) and some immunomodulators (β-glucan and others) are separately listed, with stem cell therapy and gene therapy also mentioned [11].

It becomes absolutely evident that employing such a complex therapy with substances of multidirectional biological action is hazardous to health, even in the event of a direct threat to human life, as the cumulative side effects may well surpass the consequences of radiation

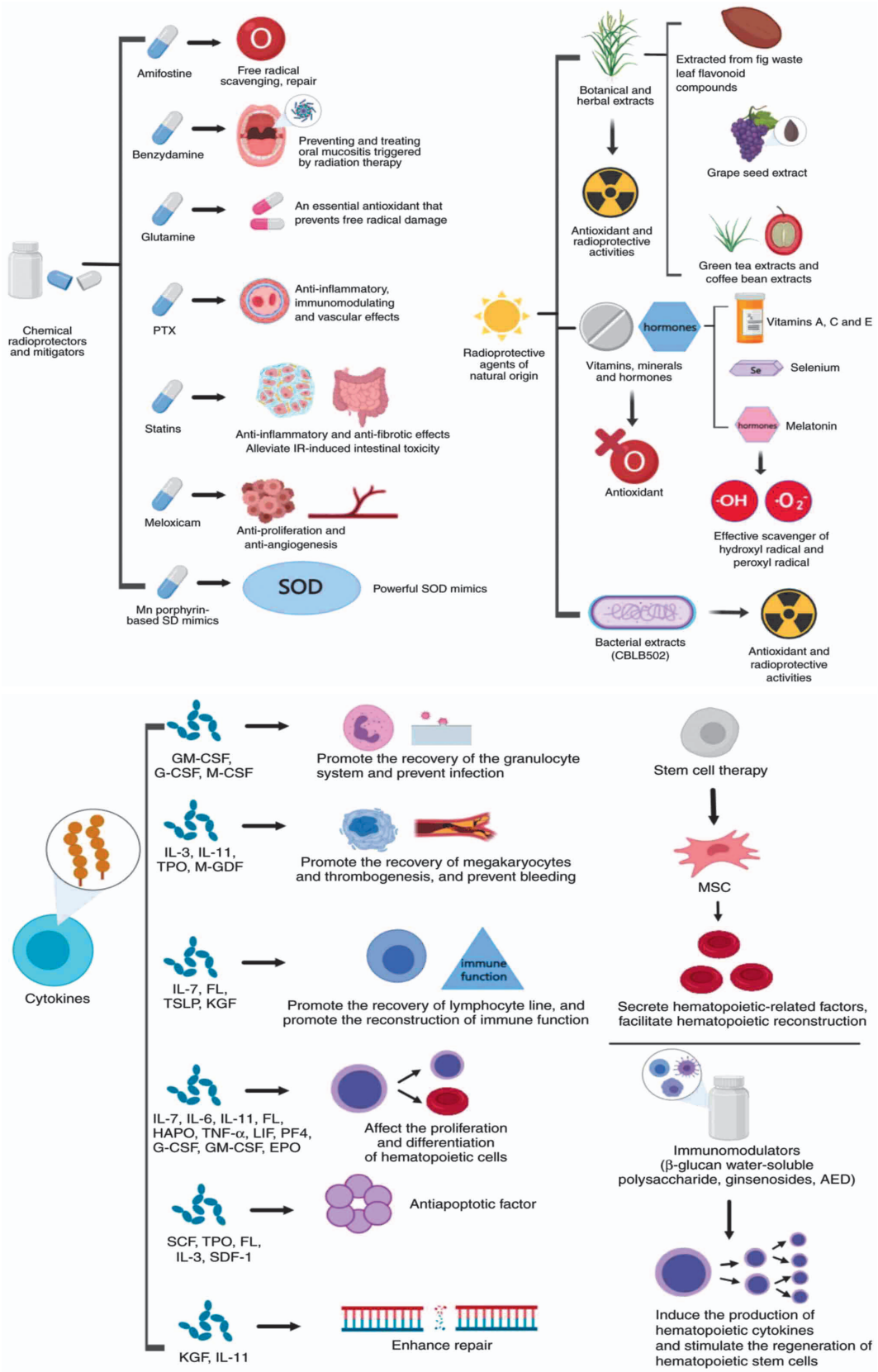

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Fig. 1. Main radioprotective agents: GM-CSF, granulocyte-macrophage colony stimulating factor; G-CSF, granulocyte colony stimulating factor; M-CSF, macrophage colony stimulating factor; IL, interleukin; TPO, thrombopoietin; M-GDF, megakaryocyte growth development factor; F L, Flt-3 ligand; TSLP, thymic stromal lymphopoietin; KGF, keratinocyte growth factor; HAPO, hemangiopoietin; LIF, leukemia inhibitor y factor; PF4, platelet factor 4; EPO, erythropoietin; SCF, stem cell factor; SDF-1, stromal cell-derived factor-1; MSC, mesenchymal stem cell; AED, 5-androstenone

exposure. This justifies the necessity of searching for more versatile medicinal methods of protection.

Properties of alpha-2-macroglobulin and prospects for its use as a means for prevention and treatment of radiation injuries

The trend toward using natural molecules with a medium or high molecular weight, which allow for a more diverse impact on the organism compared to low-molecular-weight chemical compounds, is evident. However, it has not reached its logical conclusion as high-molecular-weight blood plasma components with radioprotective properties are now rarely discussed in the scientific literature.

Meanwhile, for over half a billion years, a family of proteins that exerts a complex effect on the body's organs and systems and possesses, among other things, pronounced radioprotective properties has existed. In humans, the main representative of this family is alpha-2-macroglobulin (α2M); its concentration in blood serum is about 2–3 g/L. In humans, this family also includes pregnancy-associated alpha-2-glycoprotein and plasma protein-A (their blood levels increase during pregnancy and in estrogen-dependent tumors, but even then, they are significantly lower than the concentration of α2M). In rodents, this family additionally includes murinoglobulins. Some authors also classify the complement components C3 and C4 as part of this superfamily.

Possessing a significant molecular weight (720 kDa), α2M can perform regulatory and transport functions in the intercellular environment. This protein is known to be biologically active; however, low-molecular-weight compounds diffuse rather slowly in the absence of fluid flow. This glycoprotein has four subunits in its structure, each containing a masked thiol ester that specifically binds a wide spectrum of proteinases while partially preserving their activity. Furthermore, the subunits have a fairly extensive hydrophobic region, which is also a binding site.

A unique property of α2M, along with its ability to form covalent and non-covalent bonds with a wide variety of compounds, consists in its capacity to change the conformation and accessibility of binding sites on its surface. A number of functions are triggered only after interaction with proteinases (an example of the α2M structure based on cryo-electron microscopy data is shown in Fig. 2). Such features determine the variability of its properties, even regarding its clearance rate from circulation; thus, the α2M complex with some cytokines can circulate in the body for a long time, while after interaction with a proteinase, the half-life of the complex is no more than 1.5 min. In general, the structure and functions of α2M, including those mentioned above, have been described in sufficient detail in the scientific literature from the 1980s to the present day [12–17].

Among the properties of this protein directly or indirectly involved in radioprotection mechanisms is the fact that α2M subunits are paired by two zinc atoms and can interact with various metals via a competitive mechanism [18]. This protein is capable of performing its functions through different types of receptors expressed by various cell types, including endocytosis receptors (the low-density lipoprotein receptor family, or so-called LRP receptors) and signaling receptors (including GRP-78, classified as a heat shock protein). It has been previously established that α2M is involved in lipid metabolism, tissue remodeling, regulation of enzymatic and antioxidant system functions, while controlling the development of an inflammatory response. The fact that α2M binds and transports many cytokines, and its synthesis is regulated by cytokines and growth factors (IL-6 stimulates synthesis, while TGF-β inhibits), as well as the circumstance that α2M influences the functions of leukocytes (primarily neutrophils), lymphocytes, and macrophages, and is actively involved in inflammatory, autoimmune, and proliferative processes [12–16], indicates that many effects of currently used radioprotectors are realized with the direct participation of this protein. Among other things,

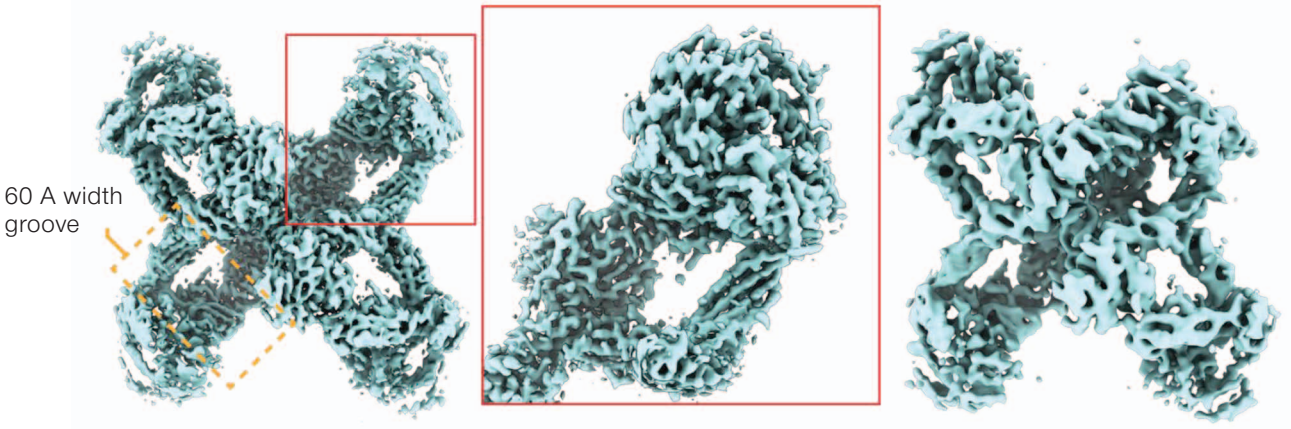

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Fig. 2. Structure of the native and transformed (after interaction with a proteinase) alpha-2-macroglobulin molecule according to cryo-electron microscopy data

α2M can induce the activation of NF-κB signaling pathways [19] and is capable of interacting with histones [20]. Despite these promising observations, only a few publications have addressed the radioprotective properties of α2M.

The first attempts found in the scientific literature to use α2M as a radioprotective agent date back to the 1960s. In 1967, the research team [21] showed that alpha-macroglobulin fractions (19S), isolated by zonal ultracentrifugation from the blood serum of rats and mice and administered both separately and in a mixture, increased the survival of mice irradiated at a dose of 750 roentgen. It was shown that the «murine» α2M fraction stimulates hematopoiesis; thus, differences in hematopoietic activity in irradiated (400 roentgen) mice receiving the macroglobulin fraction compared to the control averaged 3–5 times in the bone marrow and 9–10 times in the spleen. At the same time, the administration of a fraction of isologous proteins with a lower molecular weight did not produce a similar effect, underscoring the importance of using precisely native, undamaged preparations of high-molecular-weight proteins.

In 1974, British researchers also reported on the ability of α2M to provide radioprotection, including as part of fractions or preparations containing IgA impurities. Mice were irradiated at a sublethal dose of 500 rad and administered α2M or its containing protein fraction containing 4 h after whole-body irradiation, with a second administration 4 days later. Human albumin was used as a reference protein. It was found that the administration of α2M contributed to an increase in the total number of leukocytes. Since an unphysiologically high dose (20 mg), on the contrary, had a suppressive effect, a dose of 5 mg was considered promising. It was suggested that earlier administration (less than 4 h after radiation exposure) would be more effective [22].

Attempts to use α2M as a radioprotective agent were also made in Russia. In particular, in 1995, a patent was registered for an invention of a method for obtaining a blood plasma fraction containing α2M and intended, among other things, for the treatment of radiation injuries [23]. Despite a rather primitive and controversial production method (the fraction is essentially a mixture of α2M and IgM), the effectiveness of the development was demonstrated in clinical studies involving cancer patients undergoing chemo- and radiotherapy. The obtained preparation was administered intramuscularly multiple times. In patients additionally receiving the preparation alongside their treatment, the frequency of leukopenia and thrombocytopenia decreased, general well-being improved, the number of inflammatory infiltrates decreased, and in some cases, a regression of metastases was observed [23].

Serbian scientists published a series of works on the radioprotective properties of α2M in 2003, 2009, and 2011. The initial experiments were conducted on rats irradiated at a dose of 6.7 Gy. Amifostine was used as a reference drug. The α2M purification method included chromatography on DEAE-cellulose and gel filtration. It was demonstrated that the prophylactic administration of α2M provided 100% protection against lethal outcome at the specified radiation dose, as well as amifostine. In that experiment, a mixture of amifostine and α2M best preserved the total number of leukocytes and platelets [24].

In another study, the same research group administered α2M in physiological saline at a dose of 4.5 mg per rat weighing 200–250 g, 30 min after irradiation at 6.7 Gy. In the untreated group, about 50% of the animals died within the 4-week observation period, while in the groups receiving amifostine or α2M, all animals survived and showed weight gain. In irradiated animals without treatment, the relative liver weight (calculated as the ratio of the organ's absolute weight to the animal's body weight) decreased. Conversely, the administration of α2M and amifostine increased the relative weight, peaking in differences at 14 days. When studying morphological changes in the liver tissues of irradiated animals, the administration of α2M and amifostine minimized damage and prevented the formation of necrotic foci [25].

It was experimentally established that both α2M and amifostine significantly reduced the number of DNA damages in irradiated animals (although not fully normalizing this indicator). A comparable influence in the direction and magnitude of amifostine and α2M action on superoxide dismutase activity, the expression of the universal transcription factor NF-κB, and changes in serum IL-6 concentration in rats upon irradiation was observed. On this basis, the authors concluded that the radioprotective efficacy of α2M results from a combination of several mechanisms of action, each with its own effectiveness. It is possible that a number of the protective effects of amifostine are due to its ability to stimulate the synthesis of α2M. Thus, α2M is a central effector of natural radioprotection, at least in rats [26, 27].

These findings are of particular interest given the toxicity of amifostine, while a number of its radioprotective effects are mediated by the activity of a non-toxic protein (α2M), whose synthesis it stimulates.

In 2018, Liu et al. conducted experiments on cell cultures to demonstrate that α2M has a beneficial effect on the differentiation and proliferation of irradiated bone tissue cells, reduced autophagy, lowered oxidative stress levels, and decreased apoptosis activity, exhibiting pronounced radioprotective effects [28]. In 2022, other Chinese researchers, Huangfu et al., published data confirming the restoration of functions and maintenance of viability in irradiated fibroblasts, as well as a reduction in oxidative stress levels under the influence of α2M. Mitochondrial damage caused by irradiation was reduced with α2M, presumably by inhibiting the loss of mitochondrial membrane potential, calcium expression, and TRPM2 [29].

The significance of α2M and its receptor LRP1 (CD91) in the progression of malignant neoplasms has been repeatedly confirmed. It is suggested that restoring α2M

homeostasis in tumors to levels characteristic of healthy tissues may suppress the tumor's ability to evade immune surveillance and promote cancer cell death [30]. Since α2M levels and the activity of LRP receptor expression are directly interrelated with the growth activity of a number of malignant tumors, organismal aging, and a general decrease in resistance to external influences, it is evident that a number of teratogenic effects observed in radiation injuries may also be regulated by influencing the content of this protein in the body.

It is noteworthy that available literature contains almost no publications from Western European and North American scientists dedicated to studying the radioprotective properties of α2M. We found only one study by US researchers, who demonstrated that individuals with high levels of α2M in their blood tolerate therapeutically prescribed irradiation more easily [31].

Among the literature reviews summarizing information on the radioprotective properties of α2M, an article by Chinese specialists described its possible mechanisms of action, including the ability to stimulate antioxidant enzyme activity, prevent the development of fibrosis, maintain homeostasis and hemodynamic equilibrium, and improve DNA repair and cell recovery processes [32].

It is important to note that one of the possible reasons for the scarcity of scientific studies on the radioprotective properties of α2M relates to the difficulty in isolating highly purified preparations of α2M from blood with preserved structure and activity. Since aggressive solvents (e.g., acetonitrile) and elution buffers with acidic pH destroy the protein's structure, chromatography methods using HPLC are hardly applicable, as well as the attempts to obtain recombinant proteins enabling the purification of α2M via affinity chromatography [33]. The most acceptable methods are gentle, multi-step preparative low-pressure chromatography techniques.

Danish specialists made a significant contribution to the study and development of methods for the preparative isolation of α2M. Between 1970 and 1990, results obtained via an extensive series of studies dedicated to this protein and other members of its family were published, including descriptions of its structure, mechanisms of interaction with receptors and ligands [34–36]. The primary method proposed for isolating α2M from blood involved the removal of plasminogen by polyethylene glycol precipitation, zinc-chelate chromatography, followed by gel filtration and concentration via ultrafiltration [37]. A study of α2M and its receptors, which requires obtaining native α2M preparations in acceptable quantities, has also been undertaken by scientists from the USA [20], Germany [13], Argentina [19], and Russia [38–40]. A patent has been registered for an invention describing two stages of zinc-chelate chromatography used to isolate this protein from blood [41]. A methodological approach for obtaining α2M preparations has been published, involving the removal of plasminogen on lysine-sepharose, polyethylene glycol precipitation, anion-exchange chromatography, zinc-chelate chromatography, and gel filtration [42]. Some authors have attempted to simplify the production methods and integrate them with general blood processing approaches, as well as to increase their safety, which is already a significant step forward [43, 44]. However, the described method of obtaining α2M from the so-called Cohn fraction IV cannot be considered perfect from the perspective of the quality of the resulting protein.

In any case, methodological approaches to obtaining preparations of native α2M require adaptation for the needs of industrial production of blood-derived drugs. However, no publications have appeared thus far on the deep processing of blood serum and plasma that would enable the production of such blood preparations, beyond the "standard" list recommended for practical use in clinical practice: albumin, protein fraction (the same albumin with impurities), a number of proteins affecting blood coagulation (fibrinogen, thrombin, antihemophilic globulin, fibrinolysin, cryoprecipitate components), and certain classes of immunoglobulins.[1]

CONCLUSION

Alpha-2-macroglobulin (α2M) is a promising radioprotective agent and a key component of innate radioresistance. The administration of this protein into the organism reduces lethality and oxidative stress levels, protects DNA from damage, mitigates the severity of leukopenia and thrombocytopenia, and decreases the number of necrotic foci. Further research into the radioprotective properties of this protein and the optimization of its isolation methods from blood for industrial-scale production are required.

References

1. Supotnitskiy MV. Nuclear war as it looks. *Journal of NBC Protection Corps*. 2023;7(3):205–36 (In Russ.). https://doi.org/10.35825/2587-5728-2023-7-3-205-235
2. Indjic DR. Remediation of the areas contaminated by depleted uranium ammunition. *Military Technical Courier*. 2021;69(1):230–52. EDN: HFRRZX
3. Cheng C, Chen L, Guo K, Xie J, Shu Y, He S, et al. Progress of uranium-contaminated soil bioremediation technology. *Journal of Environmental Radioactivity*. 2022;241:106773. https://doi.org/10.1016/j.jenvrad.2021.106773
4. Gladkikh VD, Balandin NV, Basharin VA, Belovolov AYu, Grebenyuk AN, Druzhkov AV, et al. *Status and prospects of development of means of prevention and treatment of*

[1] Beloborodov VA, Kelchevskaya EA. Transfusion of blood and its components: A Study Guide. Irkutsk State Medical University of the Ministry of Health of the Russian Federation. Irkutsk: ISMU; 2020 (In Russ.).

radiation injuries. Ed. of Gladkikh VD. Moscow: Commentary; 2017 (In Russ.).
5. Grebenyuk AN, Gladkikh VD. Modern Condition and prospects of medicines for prevention and early treatment of radiation injures. *Radiation Biology. Radioecology.* 2019;59(2):132–49 (In Russ.). https://doi.org/10.1134/S0869803119020085
6. Vasin MV. B-190 (indralin) in the light of history of formation of ideas of the mechanism of action of radioprotectors. *Radiation Biology. Radioecology* 2020;60(4):378–95 (In Russ.). https://doi.org/10.31857/S0869803120040128
7. Zhang J, Li K, Zhang Q, Zhu Z, Huang G, Tian H. Polycysteine as a new type of radio-protector ameliorated tissue injury through inhibiting ferroptosis in mice. *Cell Death and Disease*. 2021;12(2):195. https://doi.org/10.1038/s41419-021-03479-0
8. Hirano S, Ichikawa Y, Sato B, Yamamoto H, Takefuji Y, Satoh F. Molecular hydrogen as a potential clinically applicable radioprotective agent. *International Journal of Molecular Sciences*. 2021;22(9):4566. https://doi.org/10.3390/ijms22094566
9. Wang H, Ahn KS, Alharbi SA, Shair OH, Arfuso F, Sethi G, et al. Celastrol alleviates gamma irradiation-induced damage by modulating diverse inflammatory mediators. *International Journal of Molecular Sciences*. 2020;21(3):1084. https://doi.org/10.3390/ijms21031084
10. Gaynutdinov TR, Ryzhkin SA, Shavaliev RF, Vagin KN, Kurbangaleev YM, Kalimullin FH, et al. Evaluation of anti-radiation efficacy of the *Staphylococcus aureus*-derived therapeutic agent. *Extreme Medicine*. 2024;6(2):63–75 (In Russ.). https://doi.org/10.47183/mes.2024.023
11. Liu L, Liang Z, Ma S, Li L, Liu X. Radioprotective countermeasures for radiation injury (Review). *Molecular Medicine Reports*. 2023;27(3):66.
12. Petersen CM. Alpha 2-macroglobulin and pregnancy zone protein. Serum level, alpha 2-macroglobuline receptors, cellular synthesis and aspects of function relation to immunology. *Danish Medical Bulletin*. 1993;40:409–46.
13. Birkenmeier G. Targetting the proteinase inhibitor and immune modulatory function of human alpha 2-macroglobulin. *Mod. Asp. Immunobiol*. 2001;2:32–6.
14. Zorin NA, Zorina VN. Macroglobulin signaling system. *Biomedical Chemistry*. 2012;58(4):400–10 (In Russ.).
15. Zorina VN, Zorin NA. Evolution and mechanisms for implementing functions of the regulatory system of proteins belonging to the macroglobulin family. *Advances in Current Biology*. 2013;133(3):284–91 (In Russ.). EDN: QYZWJN
16. Vandooren J, Itoh Y. Alpha-2-Macroglobulin in inflammation, immunity and infections. *Frontiers in Immunology*. 2021;12:803244. https://doi.org/10.3389/fimmu.2021.803244
17. Arimura Y, Funabiki H. Structural mechanics of the Alpha-2-Macroglobulin transformation. *Journal of Molecular Biology*. 2022;434(5):167413. https://doi.org/10.1016/j.jmb.2021.167413
18. Zorina VN, Evdokimova EA, Rejniuk VL. Assessing the possibility of interactions of various metals with alpha-2-macroglobulin and other human blood proteins *in vitro*. *Extreme Medicine*. 2023;25(2):105–11 (In Russ.). https://doi.org/10.47183/mes.2023.011
19. Cáceres LC, Bonacci GR, Sánchez MC, Chiabrando GA. Activated α(2) macroglobulin induces matrix metalloproteinase 9 expression by low-density lipoprotein receptor-related protein 1 through MAPK-ERK1/2 and NF-κB activation in macrophage-derived cell lines. *Journal of Cellular Biochemistry.* 2010;111(3):607–17. https://doi.org/10.1002/jcb.22737
20. Chu CT, Howard GC, Misra UK, Pizzo SV. Alpha 2-macroglobulin: a sensor for proteolysis. *Annals of the New York Academy of Sciences*. 1994;737:291–307. https://doi.org/10.1111/j.1749-6632.1994.tb44319.x
21. Hanna MG, Nettesheim P, Fisher WD, Peters LC, Francis MW. Serum alpha globulin fraction: survival-and-recovery effect in irradiated mice. *Science*. 1967;157(3795):1458–61.
22. Tunstall AM, James K. The effect of human alpha 2-macroglobulin on the restoration of humoral responsiveness in x-irradiated mice. *Clinical and Experimental Immunology*. 1975;21(1):173–80.
23. Tereshchenko IP, Murashova NS, Shalnova GA. *Method for obtaining a substance for the treatment of tumors, radiation injuries and toxic infectious conditions*. Patent of the Russian Federation No. 2042953; 1995 (In Russ.). EDN: CJUSLD
24. Sevaljević L, Dobrić S, Bogojević D, Petrović M, Koricanać G, Vulović M, Kanazir D, Ribarac-Stepić N. The radioprotective activities of turpentine-induced inflammation and alpha2-macroglobulin: the effect of dexamethasone on the radioprotective efficacy of the inflammation. *Journal of Radiation Research*. 2003;44(1):59–67. https://doi.org/10.1269/jrr.44.59
25. Mihailović M, Milosević V, Grigorov I, Poznanović G, Ivanović-Matić S, Grdović N, et al. The radioprotective effect of alpha2-macroglobulin: a morphological study of rat liver. *Medical Science Monitor*. 2009;15(7):BR188–93.
26. Mihailović M, Dobrić S, Poznanović G, Petrović M, Uskoković A, Arambasić J, et al. The acute-phase protein alpha2-macroglobulin plays an important role in radioprotection in the rat. *Shock*. 2009;31(6):607–14. https://doi.org/10.1097/shk.0b013e31818bb625
27. Bogojević D, Poznanović G, Grdović N, Grigorov I, Vidaković M, Dinić S, et al. Administration of rat acute-phase protein alpha(2)-macroglobulin before total-body irradiation initiates cytoprotective mechanisms in the liver. *Radiation and Environmental Biophysics*. 2011;50(1):167–79. https://doi.org/10.1007/s00411-010-0331-z
28. Liu Y, Cao W, Kong X, Li J, Chen X, Ge Y, et al. Protective effects of alpha-2-macroglobulinon human bone marrow mesenchymal stem cells in radiation injury. *Molecular Medicine Reports*. 2018;18(5):4219–28. https://doi.org/10.3892/mmr.2018.9449
29. Huangfu C, Tang N, Yang X, Gong Z, Li J, Jia J, et al. Improvement of irradiation-induced fibroblast damage by alpha2-macroglobulin through alleviating mitochondrial dysfunction. *Pharmaceutical Biology*. 2022;60(1):1365–73. https://doi.org/10.1080/13880209.2022.2096077
30. Olbromski M, Mrozowska M, Piotrowska A, Kmiecik A, Smolarz B, Romanowicz H, et al. Prognostic significance of alpha-2-macrglobulin and low-density lipoprotein receptor-related protein-1 in various cancers. *American Journal of Cancer Research*. 2024;14(6):3036–58. https://doi.org/10.62347/VUJV9180
31. von Reibnitz D, Yorke ED, Oh JH, Apte AP, Yang J, Pham H, et al. Predictive modeling of thoracic radiotherapy toxicity and the potential role of serum alpha-2-macroglobulin. *Frontiers in Oncology*. 2020;10:1395. https://doi.org/10.3389/fonc.2020.01395
32. Chen X, Kong X, Zhang Z, Chen W, Chen J, Li H, et al. Alpha-2-macroglobulin as a radioprotective agent: a review. *Clinical Journal of Cancer Research*. 2014;26(5):611–21. https://doi.org/10.3978/j.issn.1000-9604.2014.09.04
33. Gupalova TV, Bormotova EA. *Use of a recombinant protein that binds albumin and immunoglobulin G for proteomics purposes*. Patent of the Russian Federation No. 2758604; 2021 (In Russ.). EDN: OINWVX

34. Sottrup-Jensen L. Alpha-macroglobulins: structure, shape, and mechanism of proteinase complex formation. *The Journal of Biological Chemistry*. 1989;264(20):11539–42.
35. Sottrup-Jensen L, Stepanik TM, Kristensen T, Wierzbicki DM, Jones CM, Lønblad PB, et al. Primary structure of human alpha 2-macroglobulin. V. The complete structure. *The Journal of Biological Chemistry*. 1984;259(13):8318–27.
36. Kristensen T, Moestrup SK, Gliemann J, Bendtsen L, Sand O, Sottrup-Jensen L. Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the alpha 2-macroglobulin receptor. *FEBS Letters*. 1990;276(1–2):151–5. https://doi.org/10.1016/0014-5793(90)80530-v
37. Sottrup-Jensen L, Petersen TE, Magnusson S. A thiol-ester in alpha 2-macroglobulin cleaved during proteinase complex formation. *FEBS Letters*. 1980;121(2):275–9. https://doi.org/10.1016/0014-5793(80)80361-9
38. Zorin NA, Zhabin SG, Chirikova TS. Changes in a2-macroglobulins during evolution. *Journal of Evolutionary Biochemistry and Physiology*. 1990;26(3):289 (In Russ.).
39. Zorin NA, Zhabin SG, Belogorlova TI, Arkhipova SV. Possible similarity between α2-macroglobulin and pregnancy-dependent α2-glycoprotein and protein A: comparative study. *Problems of Medical Chemistry*. 1991;37:48–50 (In Russ.).
40. Doropheikov VV, Freidlin TS, Shcherbak IG. Human alpha-2-macroglobulin as a main cytokine-binding plasma protein. *Medical Immunology*. 1999;1(5):5–12 (In Russ.).
41. Zorin NA, Zhabin SG. *Method for isolation of alpha-macroglobulin and alpha-glycoprotein associated with pregnancy from blood plasma*. Patent of the Russian Federation No. RU 2000809; 1993 (In Russ.). EDN: CTLDGH
42. Zorin NA, Zorina RM, Zorina VN. Production of alpha-2-macroglobulin preparations with desired properties. *Russian Journal of Hematology and Transfusiology.* 2000;5:20–1 (In Russ.).
43. Huangfu C, Ma Y, Lv M, Jia J, Zhao X, Zhang J. Purification of alpha2-macroglobulin from Cohn Fraction IV by immobilized metal affinity chromatography: A promising method for the better utilization of plasma. *Journal of Chromatography B*. 2016;1025:68–75. https://doi.org/10.1016/j.jchromb.2016.05.013
44. Huangfu C, Zhao X, Lv M, Jia J, Zhu F, Wang R, et al. Inactivation of viruses during a new manufacturing process of alpha2-macroglobulin from Cohn Fraction IV by dry-heat treatment. *Transfusion*. 2016;56(9):2274–7. https://doi.org/10.1111/trf.13714

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SAFETY AND EFFICACY OF SMALL INTERFERING RNA AGENTS (LUMASIRAN) IN THERAPY FOR PRIMARY HYPEROXALURIA TYPE 1: A SYSTEMATIC REVIEW

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Introduction. Primary hyperoxaluria type 1 (PH1) is an inherited disorder characterized by excessive oxalate production in the liver, leading to hyperoxaluria, kidney stone formation, nephrocalcinosis, and progressive kidney damage. PH1 is caused by mutations in the *AGXT* gene, whereas types 2 and 3 are associated with mutations in *GRHPR* and *HOGA1*, respectively. Lumasiran, an RNA interference (RNAi)-based therapeutic agent, targets the *HAO1* gene (hydroxyacid oxidase 1), thus reducing the levels of glycolate oxidase. This action results in decreased hepatic oxalate production.

Objective. Evaluation of the efficacy, safety, and clinical use of lumasiran in adults and children with genetically confirmed primary hyperoxaluria type 1.

Materials and methods. The systematic review was conducted in accordance with the PRISMA 2020 guidelines. A comprehensive literature search was performed across four databases (PubMed, Scopus, Web of Science, and EMBASE). Studies were selected based on their focus on the use of lumasiran in pediatric or adult patients with genetically confirmed primary hyperoxaluria type 1. The quality and risk of bias were assessed using the Joanna Briggs Institute (JBI) critical appraisal tools. The final analysis included 11 studies: two randomized controlled trials, two prospective single-arm studies, one case series (involving five patients), and six individual clinical case reports involving both pediatric and adult populations.

Discussion. Lumasiran treatment was found to lead to a significant reduction in urinary oxalate (UOx) levels (approximately 60–75%) and plasma oxalate (POx) levels (approximately 30–60%). Patients across all age groups, from infants to adults, exhibited markedly stabilized or improved renal function, alongside reduced progression of nephrocalcinosis. Lumasiran demonstrated a favorable safety profile, with the most common adverse events being mild injection-site reactions. No serious treatment-related adverse events requiring discontinuation of therapy were reported.

Conclusions. By suppressing glycolate oxidase expression, lumasiran has consistently demonstrated significant efficacy in reducing oxalate levels. However, there exist differences in therapeutic approaches for adult patients and infants, as well as in treatment effects based on baseline renal function and dosing regimens. Both pediatric and adult populations showed substantial improvement and stabilization of renal function, although infants and patients with advanced chronic kidney disease required dose adjustments. Studies also revealed a greater variability in renal outcomes, particularly regarding the progression of nephrocalcinosis. Although additional large-scale long-term studies are needed, our findings indicate that lumasiran may impede the progression of kidney disease and potentially reduce or delay the need for kidney transplantation in PH1.

Keywords: primary hyperoxaluria type 1; lumasiran; small interfering RNA; pediatric patients; adult patients; oxalate; kidney injury

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БЕЗОПАСНОСТЬ И ЭФФЕКТИВНОСТЬ ТЕРАПИИ ПЕРВИЧНОЙ ГИПЕРОКСАЛУРИИ 1-ГО ТИПА С ИСПОЛЬЗОВАНИЕМ МАЛЫХ ИНТЕРФЕРИРУЮЩИХ РНК-АГЕНТОВ (ЛУМАСИРАН): СИСТЕМАТИЧЕСКИЙ ОБЗОР

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Введение. Первичная гипероксалурия 1-го типа (ПГ1) — наследственное заболевание, вызывающее избыточную выработку оксалатов в печени, что приводит к гипероксалурии, образованию камней в почках, нефрокальцинозу и прогрессирующему повреждению почек. В основе ПГ1 лежат мутации гена *AGXT*, в то время как 2-й и 3-й типы гипероксалурии вызваны мутациями *GRHPR* и *HOGA1* соответственно. Лумасиран, препарат на основе РНК-интерференции (RNAi), воздействует на ген *HAO1* (оксидаза гидроксикислот 1) и снижает уровень гликолатоксидазы, что приводит к снижению выработки оксалатов печенью.

Цель. Оценка эффективности, безопасности и особенностей клинического применения лумасирана у взрослых и детей с генетически подтвержденной первичной гипероксалурией 1-го типа.

Материалы и методы. Систематический обзор проведен согласно критериям PRISMA 2020; выполнен поиск в четырех базах данных (PubMed, Scopus, Web of Science и EMBASE). Отобраны исследования о применении лумасирана у детей или взрослых пациентов с генетически подтвержденной первичной гипероксалурией 1-го типа. Качество и риск системной ошибки оценивали с помощью инструментов критического анализа JBI (Института Джоанны Бриггс). В работу включено 11 исследований (2 рандомизированных контролируемых исследования, 2 проспективных несравнительных исследования с одной группой, 1 серия случаев (с участием 5 пациентов) и 6 индивидуальных отчетов о клинических случаях с участием детей и взрослых).

Обсуждение. Установлено, что применение лумасирана способствовало снижению уровней оксалатов в моче (UOx) (примерно на 60–75%) и оксалатов плазмы крови (POx) (примерно на 30–60%). У пациентов разного возраста, от младенцев до взрослых, значительно стабилизировалась или улучшалась функция почек и снижалось прогрессирование нефрокальциноза. Лумасиран продемонстрировал благоприятный профиль безопасности, при этом наиболее частыми побочными эффектами были слабые реакции в месте инъекции и серьезных проблем, требующих прекращения лечения, не возникало.

Выводы. Подавляя экспрессию гликолатоксидазы, лумасиран неизменно демонстрировал выраженную эффективность в снижении уровня оксалатов, однако есть различия в терапевтических подходах применения препарата у взрослых пациентов и младенцев, а также различные эффекты от воздействия в зависимости от исходной ренальной функции и режимов дозирования. Как у детей, так и у взрослых наблюдали значительное улучшение и нормализацию почечной функции, но младенцам и пациентам с прогрессирующей хронической болезнью почек требовалась корректировка дозы; в исследованиях также продемонстрирована большая вариабельность в значениях ренальных показателей и особенно в отношении прогрессирования нефрокальциноза. Хотя необходимы дополнительные крупномасштабные долгосрочные исследования, наши результаты показывают, что лумасиран может замедлять прогрессирование заболевания почек и потенциально снижать или отсрочить необходимость в трансплантации почек при ПГ1.

Ключевые слова: первичная гипероксалурия 1-го типа; лумасиран; малая интерферирующая РНК; пациенты детского возраста; взрослые пациенты; оксалат; повреждение почек

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INTRODUCTION

Primary hyperoxaluria (PH) is a rare (orphan) genetically determined autosomal recessive disorder. Its pathogenesis is rooted in impaired hepatic glyoxylate metabolism caused by mutations in the *AGXT*, *GRHPR*, and *HOGA1* genes, which encode enzymes involved in glyoxylate metabolism [1, 2].

Specifically, primary hyperoxaluria type 1 (PH1) is caused by mutations in the *AGXT* gene, encoding the enzyme alanine-glyoxylate aminotransferase (AGT). AGT deficiency or dysfunction leads to excessive conversion of glyoxylate to oxalate. This disorder results in:

- overproduction of oxalate in the liver;
- elevated plasma oxalate levels;
- increased urinary oxalate excretion;
- formation of renal calcium-oxalate crystals and radiopaque stones (primarily calcium oxalate monohydrate).

Among the main clinical manifestations are:

- kidney stone formation;
- nephrocalcinosis;
- progressive chronic kidney disease (CKD).

Without intervention, systemic oxalate deposition may occur, potentially leading to end-stage renal disease (ESRD) [3–6].

Due to critical impairments in hepatic enzymatic function and renal excretory capacity, patients with advanced PH1 may require simultaneous or sequential combined liver and kidney transplantation [7]. Therapeutic options for PH1 have conventionally been limited to conservative medical management, including high fluid intake, vitamin B6 (pyridoxine) supplementation, and crystallization inhibitors (e.g., citrate). However, these measures often fail to halt the relentless progression to end-stage renal disease (ESRD) [8].

Isolated kidney transplantation is generally insufficient, as persistent hepatic oxalate production leads to recurrent oxalate nephropathy. Consequently, combined liver-kidney transplantation has become the preferred treatment strategy [3]. Nevertheless, transplantation carries inherent surgical risks, potential graft failure, and immunological complications.

Lumasiran, a small interfering RNA (siRNA)-based therapeutic agent, received approval from the U.S. Food and Drug Administration (FDA) in November 2020 as the first drug indicated for the treatment of primary hyperoxaluria type 1 in adults and children aged six years and older [9]. OxlumoTM (lumasiran) operates via the molecular mechanism of RNA interference (RNAi), inducing degradation of target messenger RNA (mRNA) within the cell cytoplasm and enabling highly specific post-transcriptional gene regulation.

This therapeutic approach utilizes small RNA molecules to suppress the expression of specific genes by binding to complementary mRNA sequences and triggering their degradation [10, 11].

Lumasiran specifically targets the *HAO1* gene, which encodes the hydroxyacid oxidase 1 (HAO1) enzyme in hepatocytes, thereby inhibiting the production of the glycolate oxidase (GO) protein [9, 12]. By suppressing GO synthesis, lumasiran reduces hepatic glyoxylate availability and consequently decreases oxalate production, ultimately preventing the accumulation of oxalate crystals in the kidneys and other organs [12].

Clinical trials have demonstrated that lumasiran is highly effective in reducing plasma and urinary oxalate levels, leading to improved renal function in patients with PH1 [13–15]. According to Garrelfs et al., a randomized, double-blind, placebo-controlled clinical trial of Oxlumo™ (lumasiran) showed a significantly greater reduction in 24-h urinary oxalate excretion — 53.5 percentage points more with lumasiran than with placebo over a 6-month treatment period. By month 6, the majority of patients receiving lumasiran achieved urinary oxalate levels within or near the normal range. Furthermore, none of the patients in the lumasiran group developed new kidney stones, whereas kidney stones were detected in 6 out of 12 patients in the placebo group. Additionally, 84% of lumasiran-treated patients exhibited a 24-h urinary oxalate excretion no more than 1.5 times the upper limit of normal by month 6, compared to the placebo group [14].

Lumasiran also demonstrated a favorable safety profile with minimal adverse effects [15]. However, studies indicate that higher doses may be required to ensure efficacy in infants, and treatment may not fully prevent the development of nephrocalcinosis in the long term [16].

While these clinical trial results are promising, further research is needed to fully understand the safety and efficacy of lumasiran for treating hyperoxaluria. These findings may contribute to revised therapeutic protocols and reduce the need for liver transplantation in patients with PH1.

The aim of this study is to evaluate the efficacy, safety, and clinical use of OxlumoTM (lumasiran) in adults and children with genetically confirmed primary hyperoxaluria type 1.

MATERIALS AND METHODS

Study design and search strategy

A systematic review of study results was conducted in accordance with the PRISMA 2020 guidelines [17]. The review protocol was not registered in PROSPERO. A literature search was performed using the PubMed, Scopus, EMBASE, and Web of Science databases. Original studies investigating the use of lumasiran in patients with a genetic or clinical diagnosis of primary hyperoxaluria type 1 were identified.

In the PubMed/Medline database, the search was conducted using Medical Subject Headings (MeSH) terms and keywords: lumasiran, RNAi, primary hyperoxaluria type 1 (PH1), excessive hepatic oxalate, glycolate oxidase inhibition, and small interfering RNA (siRNA). To enhance search efficiency, Boolean operators OR (any of the keywords) and AND (all keywords combined) were used when combining MeSH terms and keywords up to May 2024.

Inclusion and exclusion criteria

The studies included in the systematic review comprised interventional studies (randomized controlled trials, non-comparative studies, and quasi-experimental designs), case series, and clinical case reports containing original data on clinical outcomes of Oxlumo™ (lumasiran) therapy in children and adults with PH1.

The studies excluded from consideration were reviews, animal studies, duplicate full-text publications, conference abstracts without data, and articles with insufficient patient information.

Subsequently, we analyzed sample sizes, patient demographic characteristics, details of PH1 diagnosis, lumasiran dosing and administration regimens, treatment duration, changes in urinary/plasma oxalate levels, renal function assessment data, and all reported adverse events. The studies encompassed patients across a wide age range, from infants (under one year) to elderly adults (50 years and older), with varying degrees of disease severity.

Study selection and data extraction

From the initial 91 articles identified from databases, 53 duplicate publications were excluded. Two independent experts screened the remaining 38 records by title and abstract, excluding 12 irrelevant publications. Two additional articles were included following a search of grey literature via Google Scholar and citation tracking. A total of 26 full-text publications were selected for evaluation, supplemented by 2 additional records from grey literature and citation sources. During screening, 15 articles were excluded due to overlapping data, one publication was a non-systematic review, and one was irrelevant to the topic. The remaining 11 studies met our inclusion criteria.

The systematic review included 11 studies:

- 2 randomized controlled trials,
- 2 non-comparative prospective single-arm studies,
- 1 case series (involving 5 patients),
- 6 individual clinical case reports.

These studies included both pediatric and adult patients, covering age groups from infants (< 1 year) to elderly adults (> 50 years), with varying degrees of PH1 severity.

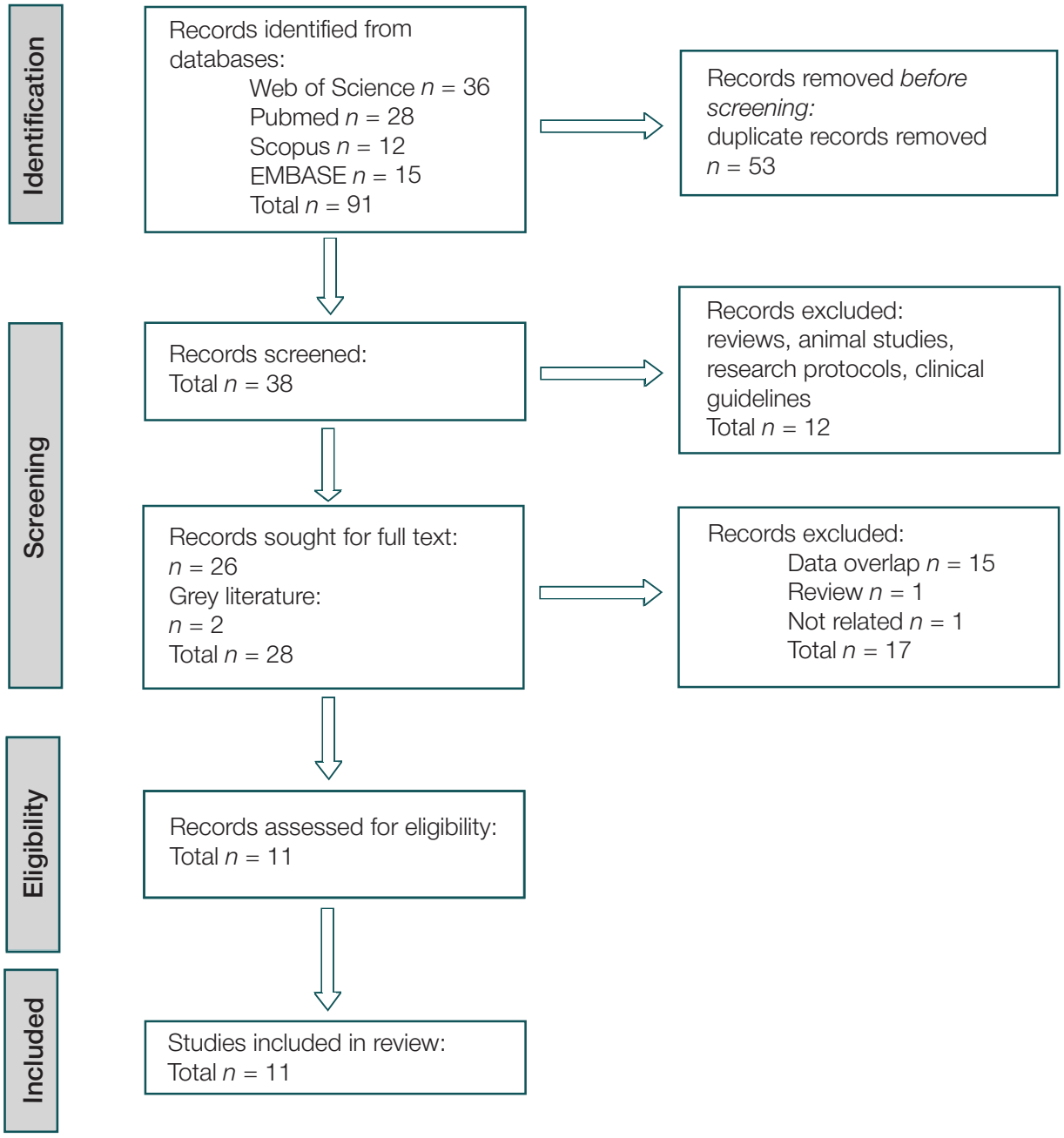

Figure prepared by the authors

Fig. PRISMA flow diagram of the systematic review

Quality assessment of included studies

The quality assessment of the included studies was conducted using the approved Joanna Briggs Institute (JBI) critical appraisal checklists, corresponding to the design of each study (randomized controlled trials, case reports, and case series studies). The criteria of each checklist were independently evaluated by two experts, and any discrepancies were resolved through consensus or consultation with a third expert. The majority of the studies demonstrated high methodological quality with minimal bias.

RESULTS AND DISCUSSION

The Table summarizes the aggregated study data, patient demographics, Oxlumo™ (lumasiran) dosing regimens, key outcomes/disease progression, and documents a comprehensive analysis of the impact of Oxlumo™ (lumasiran) on the PH1course.

Frishberg et al. [18] reported a significant reduction in mean maximum 24-h Urinary oxalate (UOx) excretion levels by 75%, or 43–92% from the baseline value of 1.69 mmol/24 h/1.73 m^2. Notably, all study participants achieved UOx levels ≤ 1.5 times the upper limit of

normal (ULN). This confirms that the core mechanism of lumasiran lies in its ability to degrade glycolate oxidase mRNA. Collectively, these results provide a comprehensive understanding of the efficacy of lumasiran in alleviating the course of PH1.

Garrelfs et al. [14] evaluated the effect of lumasiran therapy on changes in 24-h urinary oxalate excretion and plasma oxalate (POx) levels in PH1 patients. The data revealed that 84% of lumasiran-treated patients achieved 24-h UOx levels ≤ 1.5 times the ULN. Furthermore, lumasiran treatment demonstrated a significant reduction in POx levels, providing compelling evidence of its established mechanism of action.

Michael et al. [13] observed that lumasiran administration led to a marked decrease in POx levels while maintaining a favorable safety profile in individuals with progressive kidney disease and PH1.

Sas et al. [19] conducted a study investigating the efficacy of lumasiran as a therapeutic agent for treating PH1 in pediatric patients. The study utilized a regimen

Table. Summary of studies included in the systematic review on the effects of Oxlumo™ (lumasiran) on primary hyperoxaluria type 1 (PH1)

Study ID	First author, country	Year of publication	Type of Study	Sample size	Application method and dosage regimen for lumasiran	Follow-up duration	Population	Outcomes
1	Michael, Israel, France, Germany, the UK, and Netherlands [13]	2023	Clinical trial single arm	Total participants: 21 people, 0–59 years	**Children weighing <10 kg:** 6 mg/kg monthly for three months (loading phase), followed by 3 mg/kg monthly (maintenance phase). **Children weighing 10–20 kg:** 6 mg/kg monthly for three months (loading phase), followed by 6 mg/kg quarterly (every 3 months) (maintenance phase). **Children weighing >20 kg:** 3 mg/kg monthly for three months (loading phase), followed by 3 mg/kg quarterly (every 3 months) (maintenance phase). All injections were administered **subcutaneously**.	6–12 months	Total number of participants: 21 patients. All patients received treatment with lumasiran in two separate cohorts: **Cohort A** (*n* = 6; 50% female, 50% male) **Cohort B** (*n* = 15; 40% female, 60% male)	A reduction in POx levels of **33.3%** and **42.4%** was observed, alongside an acceptable safety profile for patients
2	Garrelfs, the Netherlands [14]	2021	RCT	Total Participants: 39 individuals, aged 6–47 years	3mg/kg monthly for 3 months. Followed by maintenance doses given once every 3 months, beginning 1 month after the last loading dose, followed for 6 months. All injections were performed subcutaneously	6 months	Total participants: 39 patients. **Lumasiran group:** *n* = 26 (31% female; 69% male). **Placebo group:** *n* = 13 (38% female; 62% male)	64% reduction in 24-hour UOx excretion (84% below 1.5 times the upper limit of normal) Reduction in POx levels eGFR remained stable Decrease in UOx/Cr ratio
3	Méaux, France [16]	2022	Case report	Total number of participants: 3	**Dosing regimen:** 6 mg/kg monthly for 3 months (loading phase), followed by a reduction to 3 mg/kg monthly (maintenance phase) for children weighing less than 10 kg. All injections were administered subcutaneously.	10 months	Infants before 2 years of age	Reduction in POx levels. Decrease in UOx/Cr ratio. Renal function remained normal

Table (continued)

Study ID	First author, country	Year of publication	Type of Study	Sample size	Application method and dosage regimen for lumasiran	Follow-up duration	Population	Outcomes
4	Frishberg, Israel, France, Germany, the UK, and the Netherlands [18]	2021	RCT	Total number of participants: 52 individuals, aged 6–64 years	**Dosing and Administration Regimens:** • 1 mg/kg once monthly; • 3 mg/kg once monthly; • 3 mg/kg every 3 months. **Observation period:** At least 12 weeks. All injections were administered subcutaneously	85 days, 197 days	Total number of participants: 52 individuals. **Healthy volunteers:** *n* = 32 • Lumasiran group: *n* = 24 (46% female; 54% male) • Placebo group: *n* = 8 (63% female; 37% male) **Patients:** *n* = 20 • Lumasiran group: *n* = 17 (71% female; 29% male) • Placebo group: *n* = 3 (33% female; 67% male)	A 75% reduction in 24-hour UOx excretion (≤ 1.5 times the upper limit of normal). A decrease in POx concentration
5	Sas, Israel, France, Germany, the UK, and the Netherlands [19]	2022	Clinical trial single arm	Total number of participants: 18, aged 0 months to 6 years	Dosing regimen for pediatric patients: • **Children weighing <10 kg:** 6 mg/kg monthly for three months (loading phase), followed by **3 mg/kg monthly** (maintenance phase). • **Children weighing 10 kg to <20 kg:** 6 mg/kg monthly for three months (loading phase), followed by **6 mg/kg quarterly** (every 3 months) (maintenance phase). • **Children weighing >20 kg:** 3 mg/kg monthly for three months (loading phase), followed by **3 mg/kg quarterly** (every 3 months) (maintenance phase). All injections were administered subcutaneously	6 months	Total number of participants: 18 patients. All patients received treatment with lumasiran. Stratified by weight group: • **<10 kg:** *n* = 3 (33% female); • **10 to <20 kg:** *n* = 12 (75% female); • **≥20 kg:** *n* = 3 (0% female). All treated patients (pooled): *n* = 18 (56% female)	A 72% reduction in UOx/Cr and a decrease in POx levels in children under 6 years of age (50% lower than 1.5 times the upper limit of normal)
6	Aldabek, the USA [20]	2022	Case report	Total participants: 2	Dosing regimen: 6 mg/kg monthly for the first 3 months (loading phase), followed by 3 mg/kg monthly (maintenance phase). All injections were administered subcutaneously	8 months	Two male twins, 12 months old	Significant improvement in symptoms

Table (continued)

Study ID	First author, country	Year of publication	Type of Study	Sample size	Application method and dosage regimen for lumasiran	Follow-up duration	Population	Outcomes
	Lombardi, France [21]	2023	Case report	Total participants: 1	Dosing regimen: 3 mg/kg monthly for 3 months (loading phase), followed by maintenance doses administered once every 3 months, starting 1 month after the last loading dose. All injections were administered subcutaneously	14 months	A male patient 51 years old	Decrease of SOx and UOx concentration, as well as a decrease in oxalate crystal deposition in the kidneys
	Sellier – Leclerc, France [22]	2023	Case series	Total number of participants: 5, aged 3–45 years	Lumasiran was administered via subcutaneous injections monthly for 3 months (loading phase), followed by maintenance dosing every 3 months. Data on the exact dosage are unavailable	13 months	Total number of participants: 5 patients. All patients received treatment with lumasiran	Reduction of POx level
7	Chiodini, Belgium [23]	2022	Case report	Total participants: 1	Dosing Regimen: 3 mg/kg monthly for 3 months (loading phase), followed by maintenance doses administered once every 3 months, starting 1 month after the last loading dose. All injections were administered subcutaneously	18 months	Patient boy, 13 years old	Reduction of POx and UOx levels to within normal range. 70% reduction in UOx/Cr ratio. eGFR remained stable (60 mL/min/1.73 m^2)
10	Joher, France [24]	2022	Case report	Total participants: 1	Lumasiran therapy before KTx	Duration not detailed	39 years old women	Normalization of SOx concentration before KTx
11	Poyah, Canada [25]	2021	Case report	Total participants: 1 (adult, ESKD, cutaneous manifestations)	Not specified	Duration not detailed	A 40-year-old female with primary hyperoxaluria (PH), suffering from end-stage renal disease (ESRD) with cutaneous manifestations	POx level decreased by 36%, but renal function did not recover; progression of extrarenal involvement with swan-neck deformity and pulmonary hypertension was observed

Table prepared by the authors using data from Ref. [13, 14, 16, 18–25]

Note: RCT — randomized controlled trial; NRCT — non-randomized controlled trial; POx — plasma oxalates; UOx / Cr — the ratio of oxalates in urine and creatinine level; UOx — urinary oxalates; SOx — serum oxalates; KTx — renal transplantation; eGFR — glomerular filtration rate.

of 4 or 6 loading doses of the drug, adjusted according to the patient weight. The results demonstrated a 72.0% reduction in the oxalate-to-creatinine excretion ratio (UOx:Cr). Furthermore, half of the patients achieved UOx:Cr values within half of the upper limit of normal (ULN). The reduction in POx reached 31.7%. To evaluate the impact of lumasiran therapy on PH1, six clinical studies were included in the analysis.

Méaux et al. [16] in their study observed three infants diagnosed with PH1. The patients received lumasiran therapy, with dosage and frequency adjusted based on the child's body weight. For the first 3 months, a dose of 6 mg/kg per month was prescribed; for infants weighing less than 10 kg, this regimen was adjusted to 3 mg/kg per month. As explained by Méaux et al., this method underscores the importance of weight-based factors in determining the appropriate lumasiran dose for infants with PH1. Patient 1 was diagnosed with PH1 prenatally because his older sister was diagnosed with stage 5 chronic kidney disease (CKD) at 4 months of age. After 10 months of observation, renal hyperechogenicity in the patient began to decrease, with preserved kidney function. Patient 2, diagnosed with PH1, was hospitalized due to acute renal failure and dehydration at 2.5 months of age. Serum creatinine levels were 243 μmol/L, blood urea nitrogen 19 mmol/L, with an estimated glomerular filtration rate (eGFR) of 8 mL/min/1.73 m^2, and UOx:Cr ratio (806 μmol/mmol) and POx (184 μmol/L), which were significantly elevated. After nine injections, the UOx:Cr ratio decreased by more than 60% — to 310 μmol/mmol, which was nearly normal. During the 10-month observation period, a sharp decline in serum creatinine levels was noted, eventually stabilizing at approximately 120 μmol/L (eGFR 20 mL/min/1.73 m^2). However, grade III nephrocalcinosis persisted. Due to the presence of grade III nephrocalcinosis at 3.5 months of age, patient 3 was enrolled in the study with a diagnosis of PH1. After one week, the UOx:Cr ratio increased to 2167 μmol/mmol from an elevated baseline of 1651 μmol/mmol, according to biochemical analysis. POx level was 36 μmol/L, accompanied by an elevated plasma glycolate level, but normal kidney function (creatinine 30 μmol/L, eGFR 77 mL/min/1.73 m^2). After the initial administration, a rapid decrease in the UOx:Cr ratio to 1640 μmol/mmol was observed. Kidney function remained stable throughout the observation period. After the fifth injection, nephrocalcinosis decreased from grade III to grade II. The results indicate that lumasiran is effective in infants, exhibiting no negative side effects. However, despite the good tolerability of lumasiran, it is not possible to completely avoid the occurrence or progression of nephrocalcinosis, especially in its severe forms, even when therapy is initiated in the early neonatal period or combined with standard approaches to treating PH1 [22].

In a study conducted by Aldabek et al. [20], the focus was on two male twin infants diagnosed with PH1 who exhibited symptoms of nephrolithiasis and nephrocalcinosis. These patients received lumasiran treatment starting at 12 months of age, with an initial dose of 6 mg/kg once monthly for the first three months, followed by an adjustment to 3 mg/kg monthly. Notably, the twin boys showed significant symptomatic improvement. Based on these positive outcomes, Aldabek et al. concluded that lumasiran is a successful treatment for pediatric PH1.

Chiodini et al. [23] observed an adolescent patient with PH1 who received lumasiran at a dose of 3 mg/kg over 18 months. The patient exhibited a rapid and sustained reduction in the UOx:Cr ratio, averaging 70% after lumasiran administration. Throughout the 18-month observation period, UOx levels remained low, nearly approaching the normal range. Additionally, a rapid decline in POx levels was observed, with an average reduction of approximately 60% following lumasiran treatment. The estimated glomerular filtration rate (eGFR) showed no significant changes over the entire treatment period, ranging from 60 mL/min/1.73 m^2 at baseline to 62 mL/min/1.73 m^2 at 18 months.

Lombardi et al. [21] studied the efficacy of lumasiran therapy in a 51-year-old patient with PH1 who experienced recurrent oxalate nephropathy after an isolated kidney transplant. The drug therapy involved subcutaneous administration of lumasiran at a dose of 3 mg/kg. A total of three-monthly injections were administered initially, followed by injections every three months. After initiating lumasiran, a reduction in serum oxalate (SOx) concentration, urinary oxalate, and renal oxalate crystal deposition was observed.

Another study conducted by Sellier-Leclerc et al. [22] included five patients with genetically confirmed PH1 who had undergone isolated kidney transplantation. The patients, with a mean age of 26 years (range 3–45 years), received lumasiran therapy for a median duration of 13 months (range 5–17 months). The results showed a consistent and significant reduction in POx levels in all patients after initiating lumasiran: from 110 (20–150) μmol/L to 53 (10–72) μmol/L at the time of kidney transplantation (KTx), and further to 7 (5–26) μmol/L at three months post-treatment ($p < 0.05$). Thus, in cases where the POx level ranges 80–90 μmol/L, the findings suggest that isolated KTx combined with lumasiran therapy may be a safe treatment option for PH1 patients with renal failure.

Joher et al. [24] reported a 39-year-old female with PH1 and a history of kidney transplantation (KTx) who had previously received lumasiran therapy. The results showed that SOx concentration normalized even before the KTx surgery. Lumasiran therapy led to favorable outcomes, including reductions in SOx, POx, 24-h UOx, and the UOx:Cr ratio. This was achieved through degradation of mRNA encoding glycolate oxidase, the enzyme regulating AGT, thereby reducing oxalate production.

In a study by Poyah [25], a clinical case of primary hyperoxaluria type 1 was described in a 40-year-old female with a history of recurrent nephrolithiasis. Lumasiran therapy was initiated 11 months after starting hemodialysis and pyridoxine treatment. After 14 months of high-intensity hemodialysis and three months of lumasiran, no signs of renal recovery were observed, and extrarenal complications worsened, including

progressive swan-neck deformities, reduced systolic heart function, and pulmonary hypertension. The patient was placed on the waiting list for combined liver–kidney transplantation.

The primary side effect associated with the use of lumasiran was mild and transient injection site reactions. Typical signs and manifestations included redness, skin discoloration, and hematoma at the injection site [18, 23, 25]. During studies, some patients experienced minor adverse effects, including fever, vomiting, rhinitis, abdominal pain, diarrhea, anemia, headache, or accidental overdose [13]. It is suggested that lumasiran does not have any clinically significant impact on laboratory results (including blood tests and liver function), ECG, or other vital signs [23]. This confirms that lumasiran therapy is a safe and effective treatment for infants, young children, and adults.

In our work, we studied the efficacy, safety, and clinical outcomes of lumasiran in the treatment of PH1. Our analysis of 11 studies, including randomized controlled trials, clinical case reports, and case series established that lumasiran, an RNA interference-based drug, significantly reduces oxalate levels in both plasma and urine, stabilizes or modestly improves renal function, and reduces nephrocalcinosis in patients of various ages, including adults and children. It was found that most patients achieved normal or near-normal oxalate levels while using the drug. The drug was generally well tolerated, with the most commonly reported side effect being mild injection site reactions. Thus, lumasiran represents a promising breakthrough in the treatment of PH1. Long-term follow-up data (>3 years) remain limited, particularly for infants, and further monitoring is essential to assess sustained efficacy and renal outcomes.

Across all the reviewed studies, lumasiran consistently demonstrated significant efficacy in reducing oxalate levels. However, variations were observed in patient age, baseline renal function, and dosing regimens. Both children and adults showed substantial improvement and normalization of renal function, although infants and patients with progressive chronic kidney disease required dose adjustments. The studies also revealed a greater variability in renal outcomes, particularly regarding the progression of nephrocalcinosis.

Lumasiran acts by suppressing the *HAO1* gene, which encodes glycolate oxidase—an enzyme involved in oxalate production [9]. Consequently, by inhibiting glycolate oxidase, the substrate required for oxalate production is reduced, while the levels of calcium glycolate, a less harmful metabolite, increase [17]. This reduction in oxalate synthesis leads to decreased oxalate levels in both blood and urine [25]. Numerous clinical trials and case reports have confirmed these effects and their clinical implications, such as improved renal function in both pediatric and adult patients with PH1 [13, 18, 25]. Pharmacokinetic studies indicate that lumasiran is rapidly absorbed and eliminated, supporting its favorable safety profile [9].

In the Phase III open-label single-arm study (ILLUMINATE-B) conducted in 2021, 18 children under six years of age with PH1 received lumasiran treatment for six months and demonstrated a rapid reduction in oxalate concentrations, ultimately reaching the upper limit of normal [18].

According to the ILLUMINATE-A study, kidney stone formation decreased after 6–12 months of lumasiran treatment in PH1 patients over six years of age [18, 11]. Urinary oxalate excretion also normalized [14].

Furthermore, the efficacy of lumasiran was evaluated in patients of various age groups and those with progressive CKD over 12 months in the ILLUMINATE-C study [13, 17]. As a result, POx concentrations were significantly reduced. This may delay the need for dialysis and transplantation in CKD patients and improve the prognosis for those who have already undergone kidney transplantation [28]. Regarding renal function, after several months of lumasiran treatment, eGFR remained stable or even improved [18, 28].

In summary, lumasiran may impede the progression to end-stage renal failure by improving kidney function [20, 23]. However, the optimal timing for initiating lumasiran remains unclear. While early treatment may help prevent the accumulation of oxalate crystals in the kidneys and other organs and slow the progression of nephrocalcinosis, it does not completely prevent these manifestations in some patients [23]. Therefore, further research is needed to clarify the goals of comprehensive therapy.

Another significant advantage of lumasiran consists in its favorable tolerability profile. The most frequently reported adverse events were transient, mild injection site reactions [14, 18]. Some patients experienced at least one manageable minor side effect, including fever, vomiting, rhinitis, abdominal pain, upper respiratory tract infection, diarrhea, anemia, ear infection, headache, or accidental overdose, all of which resolved rapidly during the study [13]. It appears promising that lumasiran has no clinically relevant impact on laboratory results (including hematological and liver function tests), electrocardiograms, or vital signs [23]. The studies [23] reported neither serious safety concerns—such as treatment discontinuation or drug-related deaths, nor worsening of severe symptoms [15]. Symptoms such as fatigue, nausea, reduced appetite, bone pain, decreased mobility, shortness of breath, renal colic, and others either improved or remained unchanged during lumasiran treatment [15].

While studies on the use of lumasiran for treating PH1 are promising, certain limitations and areas requiring further investigation remain. A notable drawback of many studies reviewed in our work is their sample size, frequently involving only one or several patients. In order to gain deeper insights into the safety and efficacy of this drug, larger randomized controlled trials are necessary. Additional research is also needed across diverse age groups, such as young children and elderly patients, as well as specific patient subgroups, including those with progressive kidney disease.

Furthermore, there is a lack of long-term data on the effects of lumasiran beyond one year, which is critical for understanding the full benefits and potential risks of

this therapy. The optimal dosage and treatment schedule have not yet been definitively established, particularly for pediatric patients. Although early initiation of treatment may prevent or reduce the development of nephrocalcinosis in some children, it does not fully eliminate the condition.

While lumasiran therapy appears to reduce oxalate deposition in the kidneys, further studies are needed to determine its impact on extrarenal oxalate deposition. Additionally, most existing studies were conducted in specialized centers using advanced PH1 treatment protocols, which may limit the generalizability of their findings.

Finally, combination therapy involving lumasiran and adjunctive medications may offer additional benefits; however, its potential remains to be elucidated. More clinical trials are required to enable meaningful meta-analyses. We therefore recommend conducting additional systematic reviews alongside meta-analyses to comprehensively evaluate the evidence.

In summary, while lumasiran demonstrates significant potential as a promising treatment for PH1, future large-scale studies or registry-based trials will be essential to confirm its efficacy, safety, and broader applicability. Key priorities include determining optimal dosing for neonates and patients with advanced-stage CKD, and evaluating whether combination therapies can reliably prevent the need for liver and kidney transplantation.

CONCLUSION

Our findings underscore the high efficacy and favorable safety profile of lumasiran, a breakthrough RNAi-based medication that reduces plasma and urinary oxalate levels, thereby preventing kidney damage in patients with primary hyperoxaluria type 1 (PH1), across both pediatric and adult populations. Lumasiran is primarily associated with mild, transient injection-site reactions and is emerging as a first-line therapy for PH1.

While lumasiran demonstrates robust oxalate-lowering effects, variations exist in its therapeutic application:

- age-based considerations: dosing and response differ between adults and infants, with weight-adjusted regimens critical for young children;
- renal function dependence: efficacy and dosing must be tailored to baseline kidney function, particularly in patients with advanced chronic kidney disease (CKD);
- heterogeneous renal outcomes: improvements in renal function are consistently observed, but variability persists in such metrics as nephrocalcinosis progression, especially in severe cases.

The results indicate that lumasiran can impede the progression of kidney disease and potentially reduce or delay the need for transplantation in PH1 patients. However, large-scale long-term studies are still needed to confirm these findings. Future research should focus on:

1) determining the optimal timing for therapy initiation, particularly during infancy;

2) evaluating the potential additive effects of combination therapies (e.g., with pyridoxine or conservative measures);

3) validating its durable benefits and safety over extended follow-up periods.

Further studies will provide evidence-based data to support broader clinical adoption of lumasiran, thereby enhancing understanding of treatment strategies, long-term prognoses, and outcomes for PH1 patients.

References

1. Huang Y, Zhu W, Zhou J, Huang Q, Zeng G. Navigating the Evolving Landscape of Primary Hyperoxaluria: Traditional Management Defied by the Rise of Novel Molecular Drugs. *Biomolecules*. 2024;14(5):511. https://doi.org/10.3390/biom14050511
2. Cellini B. A molecular journey on the pathogenesis of primary hyperoxaluria. *Current Opinion in Nephrology and Hypertension*. 2024;33(4):398–404. https://doi.org/10.1097/MNH.0000000000000987
3. Demoulin N, Aydin S, Gillion V, Morelle J, Jadoul M. Pathophysiology and Management of Hyperoxaluria and Oxalate Nephropathy: A Review. *American Journal of Kidney Diseases: The Official Journal of the National Kidney Foundation*. 2022;79(5):717–27. https://doi.org/10.1053/j.ajkd.2021.07.018
4. Cochat P, Hulton SA, Acquaviva C, Danpure CJ, Daudon M, De Marchi M, et al. Primary hyperoxaluria Type 1: indications for screening and guidance for diagnosis and treatment. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association — European Renal Association*. 2012;27(5):1729–36. https://doi.org/10.1093/ndt/gfs078
5. Amoroso A, Pirulli D, Florian F, Puzzer D, Boniotto M, Crovella S, et al. AGXT gene mutations and their influence on clinical heterogeneity of type 1 primary hyperoxaluria. *JASN*. 2001;12(10):2072–9. https://doi.org/10.1681/ASN.V12102072
6. Hoppe B. An update on primary hyperoxaluria. *Nature reviews. Nephrology*. 2012;8(8):467–75. https://doi.org/10.1038/nrneph.2012.113
7. Devresse A, Cochat P, Godefroid N, Kanaan N. Transplantation for Primary Hyperoxaluria Type 1: Designing New Strategies in the Era of Promising Therapeutic Perspectives. *Kidney International Reports*. 2020;5(12):2136–45. https://doi.org/10.1016/j.ekir.2020.09.022
8. Gupta A, Somers MJG, Baum MA. Treatment of primary hyperoxaluria type 1. *Clinical Kidney Journal*. 2022;15(Suppl. 1):i9–13. https://doi.org/10.1093/ckj/sfab232
9. Scott LJ, Keam SJ. Lumasiran: First Approval. *Drugs*. 2021;81(2):277–82. https://doi.org/10.1007/s40265-020-01463-0
10. Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y, et al. Therapeutic siRNA: state of the art. *Signal Transduction and Targeted Therapy*. 2020;5(1):101. https://doi.org/10.1038/s41392-020-0207-x

11. D'Ambrosio V, Ferraro PM. Lumasiran in the Management of Patients with Primary Hyperoxaluria Type 1: From Bench to Bedside. *International Journal of Nephrology and Renovascular Disease*. 2022;15:197–206. https://doi.org:10.2147/IJNRD.S293682
12. Hulton SA. Lumasiran: expanding the treatment options for patients with primary hyperoxaluria type 1. *Expert Opinion on Orphan Drugs*. 2021;9(7–10):189–98. https://doi.org/10.1080/21678707.2021.2003779
13. Michael M, Groothoff JW, Shasha-Lavsky H, Lieske JC, Frishberg Y, Simkova E, et al. Lumasiran for Advanced Primary Hyperoxaluria Type 1: Phase 3 ILLUMINATE-C Trial. *American Journal of Kidney Disease*. 2023;81(2):145–55.e1. https://doi.org:10.1053/j.ajkd.2022.05.012
14. Garrelfs SF, Frishberg Y, Hulton SA, Koren MJ, O'Riordan WD, Cochat P, et al. ILLUMINATE-A Collaborators. Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1. *The New England Journal of Medicine*. 2021;384(13):1216–26. https://doi.org/10.1056/NEJMoa2021712
15. Hulton SA, Groothoff JW, Frishberg Y, Koren MJ, Overcash JS, Sellier-Leclerc AL, et al. Randomized Clinical Trial on the Long-Term Efficacy and Safety of Lumasiran in Patients with Primary Hyperoxaluria Type 1. *Kidney International Reports*. 2022;7(3):494–506. https://doi.org/10.1016/j.ekir.2021.12.001
16. Méaux MN, Sellier-Leclerc AL, Acquaviva-Bourdain C, Harambat J, Allard L, Bacchetta J. The effect of lumasiran therapy for primary hyperoxaluria type 1 in small infants. *Pediatric Nephrology*. 2022;37(4):907–11. https://doi.org/10.1007/s00467-021-05393-1
17. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. https://doi.org/10.1136/bmj.n71
18. Frishberg Y, Deschenes G, Groothoff JW, Hulton SA, Magen D, Harambat J, et al. Phase 1/2 Study of Lumasiran for Treatment of Primary Hyperoxaluria Type 1: A Placebo-Controlled Randomized Clinical Trial. *Clinical Journal of the American Society of Nephrology*. 2021;16(7):1025–36. https://doi.org/10.2215/CJN.14730920
19. Sas DJ, Magen D, Hayes W, Shasha-Lavsky H, Michael M, Schulte I, et al. Phase 3 trial of lumasiran for primary hyperoxaluria type 1: A new RNAi therapeutic in infants and young children. *Genetics in Medicine: Official Journal of the American College of Medical Genetics*. 2022;24(3):654–62. https://doi.org/10.1016/j.gim.2021.10.024
20. Aldabek K, Grossman OK, Al-Omar O, Fox JA, Moritz ML. Infantile Primary Hyperoxaluria Type 1 Treated With Lumasiran in Twin Males. *Cureus*. 2022;14(1):e21673. https://doi.org/10.7759/cureus.21673
21. Lombardi Y, Isnard P, Chavarot N, Chauvet S, Martinez F, Thervet É, et al. Stiripentol and Lumasiran as a Rescue Therapy for Oxalate Nephropathy Recurrence After Kidney Transplantation in an Adult Patient With Primary Hyperoxaluria Type 1. *American Journal of Kidney Diseases*. 2023;82(1):113–6.10. https://doi.org/10.1053/j.ajkd.2022.12.005
22. Sellier-Leclerc AL, Metry E, Clave S, Perrin P, Acquaviva-Bourdain C, Levi C, et al. Isolated kidney transplantation under lumasiran therapy in primary hyperoxaluria type 1: a report of five cases. *Nephrology Dialysis Transplantation*. 2023;38(2):517–21. https://doi.org/10.1093/ndt/gfac295
23. Chiodini B, Tram N, Adams B, Hennaut E, Lolin K, Ismaili K. Case Report: Sustained Efficacy of Lumasiran at 18 Months in Primary Hyperoxaluria Type 1. *Frontiers in Pediatrics*. 2021;9:791616. https://doi.org/10.3389/fped.2021.791616
24. Joher N, Moktefi A, Grimbert P, Pagot E, Jouan N, El Karoui K, et al. Early post-transplant recurrence of oxalate nephropathy in a patient with primary hyperoxaluria type 1, despite pretransplant lumasiran therapy. *Kidney International*. 2022;101(1):185–6. https://doi.org/10.1016/j.kint.2021.10.022
25. Poyah P, Bergman J, Geldenhuys L, Wright G, Walsh NM, Hull P, et al. Primary Hyperoxaluria Type 1 (PH1) Presenting With End-Stage Kidney Disease and Cutaneous Manifestations in Adulthood: A Case Report. *Canadian Journal of Kidney Health Diseases*. 2021;8. https://doi.org/10.1177/20543581211058931
26. Garrelfs S, Frishberg Y, Hulton S, Koren M, O'Riordan W, Cochat P, et al. LB002 ILLUMINATE-A, a phase 3 study of lumasiran, an investigational RNAI therapeutic, in children and adults with primary hyperoxaluria type 1 (PH1). *Nephrology Dialysis Transplantation*. 2020;35(Supplement 3):gfaa146.LB002. https://doi.org/10.1093/ndt/gfaa146.LB002

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STUDY OF HORMONAL STATUS AND BONE METABOLISM IN UNDERAGE FEMALE ATHLETES WITH PRIMARY AMENORRHEA

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Introduction. Fractures, particularly low-energy ones, are more common in female athletes with oligo/amenorrhea compared to their peers without menstrual disorders. This problem is associated with various hormonal changes and impaired bone remodeling processes.
Objective. Assessment of bone metabolism and serum hormonal parameters in highly qualified under-18 female athletes both with primary amenorrhea and without menstrual cycle disorders.
Materials and methods. A single-center single-stage study involved 111 young female athletes aged 15–18 years (median age 15.9 [14.9; 16.6] years), who were members of Russian national teams in five sports. All the participants underwent comprehensive medical examination at the Federal Scientific and Clinical Center for Children and Adolescents of FMBA of Russia between March 2021 and July 2023. The athletes were divided into two groups based on the presence of primary amenorrhea. The group with primary amenorrhea included 23 athletes (median age 15.8 [15.1; 16.3] years); the comparison group consisted of 88 athletes (median age 15.9 [14.9; 16.6] years) with a regular menstrual cycle. Serum levels of osteocalcin, C-terminal telopeptide (β-CrossLaps), type 1 procollagen (P1NP), parathyroid hormone (PTH), vitamin D (25(OH)D3), and alkaline phosphatase (ALP) activity were measured. To assess hormonal status, levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and leptin were evaluated. Sexual maturity was assessed according to the Tanner rating, and body composition was evaluated using bioelectrical impedance analysis. Statistical data processing was performed using the Statistica v. 10.0 software package (StatSoft Inc., USA).
Results. Athletes with primary amenorrhea were characterized by lower body weight ($p < 0.0001$) and body fat percentage ($p < 0.0001$) compared to their peers without menstrual disorders. The analysis of LH ($p = 0.328$) and FSH ($p = 0.069$) levels did not reveal statistically significant differences between the study groups; however, the adolescent athletes with primary amenorrhea had lower levels of estradiol 182.0 [123.0; 227.0] and 244.0 [143.5; 518.5] ($p = 0.002$) and leptin 2.1 [1.2; 4.1] and 9.1 [5.1; 14.9] ($p < 0.0001$) compared those without menstrual cycle disorders. The athletes with primary amenorrhea showed an increase in both bone formation markers (P1NP, osteocalcin) and bone resorption markers (β-CrossLaps and ALP) compared to their peers without menstrual disorders.
Conclusions. Minors with primary amenorrhea are characterized by disharmonious physical development due to underweight, accompanied by reduced body fat content, decreased levels of leptin and estradiol, preserved gonadostat function, and increased markers of bone metabolism. The identified hormonal and metabolic features may represent a significant risk for impaired bone remodeling in this group of athletes.

Keywords: young athletes; sports medicine; primary amenorrhea; leptin; hormones; bone metabolism markers; vitamin D

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ИЗУЧЕНИЕ ГОРМОНАЛЬНОГО СТАТУСА И МЕТАБОЛИЗМА КОСТНОЙ ТКАНИ У НЕСОВЕРШЕННОЛЕТНИХ СПОРТСМЕНОК С ПЕРВИЧНОЙ АМЕНОРЕЕЙ

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Введение. Переломы, в особенности низкоэнергетические, чаще встречаются у спортсменок с олиго/аменореей по сравнению со сверстницами без нарушений менструального цикла, что связано с различными гормональными изменениями и нарушением процессов ремоделирования костной ткани.
Цель. Оценка состояния метаболизма костной ткани и гормональных показателей в сыворотке крови у высококвалифицированных спортсменок, не достигших 18-летнего возраста, с первичной аменореей и без нарушений менструального цикла.

Материалы и методы. Проведено одномоментное одноцентровое исследование с участием 111 юных спортсменок в возрасте 15–18 лет (средний возраст 15,9 [14,9; 16,6] года), входящих в состав сборных команд РФ по 5 видам спорта и проходивших углубленное медицинское обследование в ФГБУ «ФНКЦ детей и подростков ФМБА России» в период с марта 2021 по июль 2023 г. Спортсменки были разделены на 2 группы в зависимости от наличия первичной аменореи. В группу с первичной аменореей включены 23 спортсменки (средний возраст 15,8 [15,1; 16,3] года); в группу сравнения — 88 спортсменок (средний возраст 15,9 [14,9; 16,6] года) с регулярным менструальным циклом. У спортсменок определяли уровень остеокальцина, С-концевого телопептида (β-CrossLaps), проколлагена 1-го типа (P1NP), паратиреоидного гормона (ПТГ), витамина D (25(OH)D3) и активности щелочной фосфатазы (ЩФ) в сыворотке крови. Для оценки гормонального статуса проведена оценка уровней лютеинизирующего гормона (ЛГ), фолликулостимулирующего гормона (ФСГ), эстрадиола и лептина. Оценка полового развития проведена по классификации Tanner, оценка композиционного состава тела — методом биоимпедансного анализа. Статистическая обработка данных произведена с использованием пакета прикладных программ Statistica v. 10.0 (StatSoft Inc., США).
Результаты. Для спортсменок с первичной аменореей характерны более низкие значения массы тела ($p < 0,0001$) и содержания жировой ткани (%) в организме ($p < 0,0001$) по сравнению со сверстницами без нарушений менструального цикла. Анализ уровней ЛГ ($p = 0,328$) и ФСГ ($p = 0,069$) не выявлял статистически значимых различий в исследуемых группах, однако у девочек с первичной аменореей отмечали более низкие уровни эстрадиола 182,0 [123,0; 227,0] и 244,0 [143,5; 518,5] ($p = 0,002$) и лептина 2,1 [1,2; 4,1] и 9,1 [5,1; 14,9] ($p < 0,0001$) по сравнению со спортсменками без нарушений менструального цикла. У спортсменок с первичной аменореей выявлено повышение как маркеров костеобразования (P1NP, остеокальцин), так и костной резорбции (β-CrossLaps и ЩФ) по сравнению со сверстницами без нарушений менструального цикла.
Выводы. Для несовершеннолетних спортсменок с первичной аменореей характерно дисгармоничное физическое развитие за счет дефицита массы тела, сопровождающееся снижением содержания жировой ткани в организме, снижением уровня лептина и эстрадиола и сохранной функцией гонадостата, а также повышением маркеров метаболизма костной ткани. Выявленные гормональные и метаболические особенности могут являться значимым фактором риска развития нарушений ремоделирования костной ткани у данной группы спортсменок.

Ключевые слова: юные спортсменки; спортивная медицина; первичная аменорея; лептин; гормоны; маркеры костного метаболизма; витамин D

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Финансирование: исследование выполнено в рамках прикладной научно-исследовательской работы «Развитие персонализированного подхода в ведении несовершеннолетних спортсменов спортивных сборных команд Российской Федерации» (шифр «Дети в спорте-25/27»).

Соответствие принципам этики: исследование одобрено этическим комитетом ФГБУ «ФНКЦ детей и подростков ФМБА России» (протокол № 1 от 13.02.2025). Родители/опекуны или законные представители спортсменов подписали добровольное информированное согласие на участие в исследовании.

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INTRODUCTION

Relative energy deficiency in sport (RED-s) in adolescent females is often associated with the development of functional hypothalamic amenorrhea (FHA) [1, 2]. Prolonged energy deficiency is accompanied by a decrease in the pulsatile secretion of gonadotropin-releasing hormone in the hypothalamus, followed by impaired release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. In turn, this leads to decreased estradiol levels and the development of menstrual disorders, such as primary and secondary amenorrhea [3]. Underweight and particularly reduced adipose tissue in athletes with long-term energy deficit are also associated with decreased leptin levels — a hormone produced by adipose tissue that is an important regulator of gonadostat functional activity [4].

The FHA development is associated with reduced bone mass accumulation and impaired bone microarchitecture, being a leading risk factor in low-energy fractures in professional female athletes, particularly those under 18 years of age [1, 5, 6].

Studies into bone metabolism markers and their relationship with hormonal parameters in underage highly trained female athletes with primary amenorrhea are limited and show contradictory results [5].

The aim of this study is to assess bone metabolism and serum hormonal parameters in highly qualified under-18 female athletes both with primary amenorrhea and without menstrual cycle disorders.

MATERIALS AND METHODS

A single-center single-stage study involved young athletes from the Russian national teams who underwent comprehensive medical examination at the Federal Scientific and Clinical Center for Children and Adolescents of FMBA between March 2021 and July 2023. A total of 111 young female athletes aged 15–18 years (median age 15.9 [14.9; 16.6] years), representing five sports (rhythmic gymnastics, artistic gymnastics, figure skating,

softball, synchronized swimming), included in the study, were divided into two groups based on the presence of primary amenorrhea. Primary amenorrhea was diagnosed based on the absence of menstruation by age 15[1] (provided that secondary sexual characteristics had developed).

The group with primary amenorrhea included 23 athletes (median age 15.8 [15.1; 16.3] years); the comparison group consisted of 88 athletes (median age 15.9 [14.9; 16.6] years) with a regular menstrual cycle. The study groups were comparable in age (p = 0.794) and sexual maturity stage, although showing statistically significant differences in the key anthropometric parameters (Table 1). Anthropometric measurements of the underage athletes included: height, body weight, and calculation of body mass index (BMI). BMI was assessed for the specific age and sex and presented as the number of standard deviations from the mean (SDS). Body composition assessment was performed using bioelectrical impedance analysis (InBody 570 analyzer, South Korea). Sexual maturity of the underage athletes was assessed according to the Tanner rating.

When assessing sexual maturity, 21 (92%) athletes in the study group and 88 (100%) athletes in the comparison group had completed or nearly completed sexual maturity. Sexual maturity was assessed according to the Tanner rating [7]. Inclusion criteria for study participants were female athletes from Russian national teams aged 15–18 years and the presence of primary amenorrhea. Inclusion criteria for the comparison group were regular menstrual cycle, gynecological age > 1 year.

For clinical and laboratory analysis, blood samples were collected from a peripheral vein in the morning after fasting. All young athletes had their serum levels of osteocalcin (Roche, Switzerland), N-terminal propeptide of human type 1 procollagen (P1NP) (Roche, Switzerland), C-terminal telopeptide (β-CrossLaps) (Roche, Switzerland), vitamin D (25-hydroxycholecalciferol-25(OH)D3) (Roche, Switzerland) measured (ng/mL). Parathyroid hormone (PTH) (Roche, Switzerland) in serum (pmol/L). Leptin (ng/mL), luteinizing hormone (LH) (IU/L), follicle-stimulating hormone (FSH) (IU/L), and estradiol (pmol/L) levels were determined by enzyme immunoassay (manufacturer Bender MedSystems, Austria). β-CrossLaps testing was performed by electrochemiluminescence using a Cobas e411 analyzer (Roche Diagnostics, Germany). Testing of P1NP, osteocalcin, PTH, 25(OH)D3 was carried out by solid-phase enzyme immunoassay. Serum alkaline phosphatase (ALP) activity (U/L) was determined by a kinetic colorimetric method.

Statistical data processing was performed using the Statistica v. 10.0 software package (StatSoft Inc., USA). Since the studied quantitative indicators had a non-normal distribution (according to the Kolmogorov–Smirnov test), all data are presented as median (M_e) and 1st and 3rd quartiles [Q_1; Q_3]. The Mann–Whitney U test was used to assess the statistical significance of differences in quantitative characteristics. Qualitative characteristics are presented as percentages (%) with absolute values. Contingency tables were constructed to assess differences between qualitative characteristics, followed by evaluation using Pearson's chi-square test (χ^2) with Yates' correction. Correlation analysis was performed using Spearman's criterion. A statistical significance level of $p \leq 0.05$ was accepted for differences.

RESULTS

Athletes with primary amenorrhea were characterized by lower parameters of height (p = 0.023), body weight

Table 1. Clinical characteristics of the study groups

Parameter	Group with primary amenorrhea (n = 23)	Group with regular menstrual cycle (n = 88)	Statistical significance level, p
Age, years	15.8 [15.1; 16.3]	15.9 [14.9; 6.6]	0.794
Height, m	1.63 [1.56; 1.67]	1.66 [1.61; 1.71]	0.023
Height SDS	0.15 [–1.17; 0.87]	0.66 [–0.06; 1.5]	0.016
Body weight, kg	46.8 [40.5; 48.8]	60.6 [54.2; 67.7]	< 0.0001
BMI	17.4 [16.6; 18.2]	21.8 [19.7; 24.0]	< 0.0001
BMI SDS	–1.34 [–1.69; –0.88]	0.5 [–0.07; 1.14]	< 0.0001
Sexual maturity: Tanner II–III Tanner IV–V	 2 (8%) 21 (92%)	 – (–) 88 (100%)	0.059

Table compiled by the authors based on their own data

Note: n = number of athletes; "–" — the absence of athletes at Tanner sexual maturity stages II–III in this group.

[1] Clinical guidelines "Amenorrhea and oligomenorrhea"; 2024 (In Russ.). URL: https://cr.minzdrav.gov.ru/preview-cr/644_2?ysclid=mdyjwyzaqy766941934

($p < 0.0001$), BMI ($p < 0.0001$), and BMI SDS ($p < 0.0001$) compared to their peers without menstrual disorders.

An analysis of gonadotropin levels (Table 2) did not reveal statistically significant differences between the study groups ($p = 0.328$ for LH; $p = 0.069$ for FSH). However, adolesent althleters with primary amenorrhea had lower levels of estradiol, 182.0 [123.0; 227.0], and leptin, 2.1 [1.2; 4.1], compared to those without menstrual cycle disorders: 244.0 [143.5; 518.5] ($p = 0.002$) and 9.1 [5.1; 14.9] ($p < 0.0001$), respectively.

A correlation analysis revealed a strong positive correlation between leptin levels and body fat percentage ($r_s = 0.74$; $p < 0.05$), LH levels ($r_s = 0.16$; $p < 0.05$), and estradiol levels ($r_s = 0.24$; $p < 0.05$).

According to the assessed bone metabolism parameters, athletes with primary amenorrhea showed an increase in both bone formation markers (P1NP by 2.5 times and osteocalcin by almost 2 times) and bone resorption markers β-CrossLaps and ALP compared to their peers without menstrual disorders; the corresponding data are presented in Table 2. The study groups did not show statistically significant differences in PTH levels ($p = 0.242$). However, when assessing vitamin D status, athletes with a regular menstrual cycle had lower levels of 25(OH)D3 compared to the primary amenorrhea group ($p = 0.001$).

An evaluation of body composition in underage athletes with primary amenorrhea revealed a statistically significant reduction in body fat percentage (%) compared to the group of athletes with a regular menstrual cycle: 10.8 [9.3; 12.8] vs. 20.5 [16.1; 24.4], ($p < 0.0001$).

The conducted correlation analysis established a moderate negative association between leptin levels and osteocalcin ($r_s = -0.33$), P1NP ($r_s = -0.39$), β-CrossLaps ($r_s = -0.45$), and ALP ($r_s = -0.43$). Meanwhile, bone metabolism markers in underage athletes were not dependent on estradiol and gonadotropin levels.

DISCUSSION

It is known that fractures, particularly low-energy ones, are more common in athletes with oligo/amenorrhea compared to their peers without menstrual disorders and with normal physical activity levels [8]. The presence of primary amenorrhea is currently considered by the International Olympic Committee expert panel as an important risk factor used for stratifying the risks of developing RED-s syndrome, including in underage athletes [1].

Analysis of bone metabolism markers is an effective diagnostic tool for assessing the functional state of the skeletal system in clinical practice [9]. Our work demonstrated that underage athletes with primary amenorrhea demonstrate elevated levels of key bone metabolism markers compared to athletes without menstrual disorders. It is known that estrogens promote the inhibition of bone resorption processes [10], and their deficiency, identified in athletes with primary amenorrhea, leads to an increase in bone resorption markers. However, our results are not consistent with the data by Christo et al., who found reduced levels of N-terminal telopeptide (NTX) and P1NP in athletes with amenorrhea and low bone mineral density (BMD). The authors explained their finding by a “slowdown” in bone metabolism due to chronic energy deficiency in athletes [5].

Some authors have shown that the presence of menstrual disorders in young athletes is accompanied by reduced BMD, as determined by X-ray densitometry

Table 2. Hormonal parameters and bone metabolism markers in underage highly qualified female athletes depending on the presence of primary amenorrhea

Studied parameters	Group with primary amenorrhea ($n = 23$)	Group with regular menstrual cycle ($n = 88$)	Statistical significance level, p
Osteocalcin, ng/mL	92.2 [60.0; 110.0]	49.0 [37.0; 65.0]	< 0.0001
P1NP, ng/mL	505.3 [406.8; 750.8]	200.7 [136.0; 244.9]	< 0.0001
ALP, U/L	200.2 [161.7; 285.1]	92.7 [75.3; 127.3]	< 0.0001
β-CrossLaps, ng/mL	1.78 [1.39; 2.11]	1.27 [0.98; 1.51]	0.0001
PTH, pmol/L	4.6 [2.7; 5.4]	5.0 [3.6; 6.6]	0.242
25(OH)D3, ng/mL	23.5 [13.3; 32.8]	14.3 [11.1; 19.8]	0.001
LH, IU/L	2.8 [2.3; 4.1]	3.4 [2.0; 5.8]	0.328
FSH, IU/L	5.4 [4.6; 6.5]	4.8 [3.5; 6.1]	0.069
Estradiol, pmol/L	182.0 [123.0; 227.0]	244.0 [143.5; 518.5]	0.002
Leptin, ng/mL	2.1 [1.2; 4.1]	9.1 [5.1; 14.9]	< 0.0001

Table compiled by the authors based on their own data

Note: n = number of athletes.

[5]. The reduction in bone tissue mineralization in highly qualified young athletes occurs despite the presence of strength and intensive physical loads, which have a protective effect on bone tissue [11].

Our work demonstrated that athletes with primary amenorrhea had lower BMI SDS values, which, according to the literature, is a predictor of reduced BMD [12]. The group of athletes with primary amenorrhea was predominantly represented by rhythmic gymnastics and figure skating. In these sports, low body weight is a key factor for success; for this reason, athletes often resort to hypocaloric unbalanced diets, which is one of the causes of developing FHA within RED-s explaining the presence of primary amenorrhea [13]. For example, in a study of athletes aged 11–17 years engaged in rhythmic gymnastics, disharmonious physical development due to underweight was revealed, accompanied by a reduction in body fat and a high prevalence of primary amenorrhea (38%) [14].

Therefore, BMI SDS and estrogen deficiency can be considered as the leading independent factors contributing to impaired mineralization and microarchitecture of bone tissue in athletes with FHA. Underweight in athletes is associated with a reduction in adipose tissue and circulating leptin. Normally, leptin, by affecting the secretory activity of gonadotrophs, increases the pulsatile secretion of LH and, to a lesser extent, FSH [15]. Currently, leptin is believed to be a crucial endogenous regulator and modulator of reproductive system functions, the dysfunction of which is a key factor in impaired bone remodeling and reduced BMD in athletes with RED-s syndrome [1, 17].

In addition to weight deficiency, athletes with menstrual disorders have been recorded to have lower height and height SDS indicators, which may be due to reduced secretion of insulin-like growth factor-1 and the development of partial resistance to growth hormone [1, 2].

An important limitation of our study is the presence of vitamin D deficiency or insufficiency in the majority of athletes in both study groups, which could have influenced the levels of the studied bone metabolism markers. We did not assess the impact of sports type on the levels of the investigated bone metabolism markers due to the small sample size of athletes with primary amenorrhea. Furthermore, underage athletes did not undergo assessment of biological (bone) age using hand radiography. It is known that bone age is an independent predictor of bone remodeling marker levels, which physiologically increase during active growth in puberty [17–19]. The greater the bone maturation values, the less growth potential the child has, and the lower the levels of bone remodeling markers he or she demonstrates.

Further studies into the characteristics of hormonal status and bone remodeling markers and their influence on BMD may have important practical significance for developing an individualized approach to diagnosing impaired bone remodeling and stratifying the risks of low-energy fractures in underage athletes with FHA.

CONCLUSION

The development of primary amenorrhea in underage highly trained athletes with FHA is accompanied by decreased estrogen levels, although not being associated with impaired gonadostat function. The state of underweight identified in athletes with primary amenorrhea is caused by a deficit in adipose tissue and is accompanied by reduced blood leptin levels, which may contribute to the progression of reproductive system disorders.

Elevated bone metabolism markers in athletes with FHA may indicate disturbances in bone remodeling processes or may reflect ongoing growth and development in adolescents. Given the known negative impact of estrogen deficiency on BMD, underage athletes with underweight and FHA constitute a high-risk group for developing low-energy fractures.

The influence of elevated bone metabolite levels in athletes with amenorrhea on bone mineral density, bone structure, and the risk of increased trauma requires further research and elucidation.

References

1. Mountjoy M, Ackerman KE, Bailey DM, Burke LM, Constantini N, Hackney AC, et al. 2023 International Olympic Committee's (IOC) consensus statement on Relative Energy Deficiency in Sport (REDs). *British Journal of Sports Medicine*. 2023;57(17):1073–97. https://doi.org/10.1136/bjsports-2023-106994
2. Hackney AC, Constantini NW. *Endocrinology of Physical Activity and Sport*. 3rd ed. Cham: Humana press; 2020. https://doi.org/10.1007/978-3-030-33376-8
3. Khosla S. Update on estrogens and the skeleton. *The Journal of Clinical Endocrinology and Metabolism*. 2010;95(8):3569–77. https://doi.org/10.1210/jc.2010-0856
4. de Assis GG, Murawska-Ciałowicz E. Exercise and weight management: the role of leptin — a systematic review and update of clinical data from 2000–2022. *Journal of Clinical Medicine.* 2023;12(12):4490. https://doi.org/10.3390/jcm12134490
5. Christo K, Prabhakaran R, Lamparello B, Cord J, Miller KK, Goldstein MA, et al. Bone metabolism in adolescent athletes with amenorrhea, athletes with eumenorrhea, and control subjects. *Pediatrics*. 2008;121(6):1127–36. https://doi.org/10.1542/peds.2007-2392
6. Kaga M, Takahashi K, Ishihara T, Suzuki H, Tanaka H, Seino Y, et al. Bone assessment of female long-distance runners. *Journal of Bone and Mineral Metabolism*. 2004;22(5):509–13. https://doi.org/10.1007/s00774-004-0515-1
7. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of Disease in Childhood*. 1976;51(3):170. https://doi.org/10.1136/adc.51.3.170
8. Ackerman KE, Cano Sokoloff N, de Nardo Maffazioli G, Clarke HM, Lee H, Misra M. Fractures in relation to menstrual status and bone parameters in young athletes. *Medicine and Science in Sports and Exercise.* 2015;47(8):1577–86. https://doi.org/10.1249/mss.0000000000000574

9. Kiseleva NG, Taranushenko TE, Golubenko NK. Diagnosis of osteoporosis at an early age. *Medical Counsil.* 2020;1:179–86 (In Russ.). https://doi.org/10.21518/2079-701X-2020-1-186-193
10. Riggs BL, Khosla S, Melton LJ. Sex steroids and the construction and conservation of the adult skeleton. *Endocrine Reviews.* 2002;23(3):279–302. https://doi.org/10.1210/edrv.23.3.0465
11. Duncan CS, Blimkie CJ, Cowell CT, Burke ST, Briody JN, Howman-Giles R. Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength. *Medicine and Science in Sports and Exercise.* 2002;34(2):286–94. https://doi.org/10.1097/00005768-200202000-00017
12. Markou KB, Mylonas P, Theodoropoulou A, Kontogiannis A, Leglise M, Vagenakis A, et al. The influence of intensive physical exercise on bone acquisition in adolescent elite female and male artistic gymnasts. *The Journal of Clinical Endocrinology and Metabolism.* 2004;89(9):4383–7. https://doi.org/10.1210/jc.2003-031865
13. Bezuglov EN, Lazarev AM, Khaitin VYu, Barskova EM, Koloda YuA. The impact of professional sports on menstrual function. *Russian Journal of Human Reproduction*. 2020;26(4):37–47 (In Russ.). https://doi.org/10.17116/repro20202604137
14. Evdokimova NV, Bodrova MV, Solovyova AS, Salikova PD, Moskvina AR. Features of physical development, menstrual function and body composition in children engaged in professional sports (rhythmic gymnastics). *Medicine: Theory and Practice.* 2024;9(2):32–9 (In Russ.). https://doi.org/10.56871/MTP.2024.58.20.004
15. Odle AK, Akhter N, Syed MM, Allensworth-James ML, Beneš H, Melgar Castillo AI, et al. Leptin regulation of gonadotrope gonadotropin-releasing hormone receptors as a metabolic checkpoint and gateway to reproductive competence. *Frontiers in Endocrinology.* 2018;8:367. https://doi.org/10.3389/fendo.2017.00367
16. Mathew H, Castracane VD, Mantzoros C. Adipose tissue and reproductive health. *Metabolism: Clinical and Experimental*. 2018;86:18–32. https://doi.org/10.1016/j.metabol.2017.11.006
17. Chubb SAP, Vasikaran SD, Gillett MJ. Reference intervals for plasma β-CTX and P1NP in children: A systematic review and pooled estimates. *Clinical Biochemistry*. 2023;118:110582. https://doi.org/10.1016/j.clinbiochem.2023.05.001
18. Bayer M. Reference values of osteocalcin and procollagen type I N-propeptide plasma levels in a healthy Central European population aged 0–18 years. *Osteoporosis International.* 2014;25(2):729–36. https://doi.org/10.1007/s00198-013-2485-4
19. Crofton PM, Evans N, Taylor MR, Holland CV. Serum CrossLaps: pediatric reference intervals from birth to 19 years of age. *Clinical Chemistry*. 2002;48(4):671–3.

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INJURIES AND DAMAGE TO LARGE JOINTS IN UNDERAGE ATHLETES. THERAPY WITH PLATELET-RICH PLASMA. A CLINICAL CASE

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Introduction. Approximately 46% of large joint injuries involve damage to the knee joint, among which anterior cruciate ligament (ACL) injuries account for about 15–24% in children and adolescents. In order to reduce the likelihood of long-term complications after injuries in underage professional athletes, shorten the rehabilitation period, and enable a quicker return to elite sports, innovative regenerative medicine technologies should be implemented into clinical practice, including the use of platelet-rich plasma (PRP). In comparison with other conservative treatment methods, PRP offers several advantages. Being an orthobiological agent, PRP is a biological substance derived from the patient's own body, promoting accelerated regeneration with minimal risk of side effects. When performed correctly, this procedure shows minimal invasiveness and does not lead to complications. Although PRP therapy is widely used in treating diseases and injuries of large joints in adult patients, the application of this therapeutic method in underage athletes has not been sufficiently studied, which is the focus of this study.
Case report. We present a clinical case of a professional athlete with a knee joint injury, assessing the functional and clinical outcomes of PRP therapy. Two standardized methods for PRP application are demonstrated, highlighting the advantages of a closed-loop PRP preparation system. These include minimized exposure to the external environment, mitigating potential risks of infection, and reduced consumption of materials, enhancing cost-effectiveness. The described clinical case of an ACL injury in a junior athlete, with complete functional and structural recovery (as confirmed by magnetic resonance imaging, MRI), demonstrated the efficacy, safety, and good tolerability of PRP therapy. Positive outcomes were observed both clinically (regression of pain assessed via the visual analog scale, restoration of joint function, and positive dynamics in provocative tests such as the Lachman test and anterior drawer test) and through MRI data.
Conclusions. The use of PRP therapy for ACL injuries in underage professional athletes represents a promising therapeutic approach in orthopedics and sports medicine, utilizing regenerative medicine technologies. The closed-loop system for PRP preparation offers several advantages over open-loop systems, including cost-effectiveness due to minimized consumption of medical supplies. The safety of the method is ensured provided that the procedural requirements are met (asepsis, antisepsis, ultrasound guidance, etc.); the high sensitivity of MRI in tracking the dynamics of ACL injuries is confirmed.

Keywords: sports medicine; pediatric sports injuries; large joint damage; PRP therapy; underage athletes

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ТРАВМЫ И ПОВРЕЖДЕНИЯ КРУПНЫХ СУСТАВОВ У НЕСОВЕРШЕННОЛЕТНИХ СПОРТСМЕНОВ. ТЕРАПИЯ ПЛАЗМОЙ, ОБОГАЩЕННОЙ ТРОМБОЦИТАМИ. КЛИНИЧЕСКИЙ СЛУЧАЙ

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Введение. Около 46% травм крупных суставов занимают повреждения коленного сустава, в структуре которых у детей и подростков порядка 15–24% приходится на переднюю крестообразную связку (ПКС). Для сокращения вероятности отдаленных осложнений после травм у несовершеннолетних профессиональных спортсменов, реабилитационного периода и скорейшего возвращения в спорт высших достижений необходимо внедрение в клиническую практику инновационных технологий регенеративной медицины, в том числе с использованием плазмы, обогащенной тромбоцитами (PRP). Применение PRP имеет ряд преимуществ по сравнению с другими консервативными методами терапии, а именно: являясь ортобиологическим препаратом, PRP представляет собой биологическое вещество собственного организма пациента, способствующее ускорению регенерации с минимальным риском возникновения побочных явлений; это малоинвазивная манипуляция, при соблюдении методики не приводящая к осложнениям. Несмотря на то что PRP-терапия широко применяется при лечении заболеваний и повреждений крупных суставов у взрослой

категории пациентов, использование данного терапевтического метода у несовершеннолетних спортсменов изучено недостаточно, в связи с чем и явилось целью настоящей работы.
Описание клинического случая. Представлено клиническое наблюдение профессионального спортсмена с травмой коленного сустава с оценкой функциональных и клинических результатов PRP-терапии. В данной работе продемонстрированы две стандартизированные методики по применению PRP, обозначены преимущества закрытого цикла приготовления PRP: минимизация контакта с внешней средой, снижающего возможные риски инфицирования; сокращение расходных материалов, повышающего экономическую эффективность. Описанный клинический случай травмы ПКС у юниора с полным функциональным и структурным (по данным магнитно-резонансной томографии) восстановлением при проведении PRP-терапии продемонстрировал эффективность, безопасность и хорошую переносимость применения плазмы, обогащенной тромбоцитами. Положительный результат отмечен как клинически (констатация регресса болевого синдрома, оцениваемого по визуально-аналоговой шкале, восстановления функции сустава, положительная динамика провокационных тестов (тест Лахмана и переднего выдвижного ящика)), так и по данным визуализации.
Выводы. Применение PRP-терапии при травмах ПКС у несовершеннолетних профессиональных спортсменов является перспективным терапевтическим подходом в ортопедии и спортивной медицине с использованием технологий регенеративной медицины. Закрытый цикл приготовления PRP имеет ряд преимуществ перед открытым, включая экономическую эффективность благодаря минимизации расхода медицинских изделий. Показана безопасность метода при соблюдении требований к его проведению (асептика, антисептика, ультразвуковой контроль и др.) и подтверждена высокая чувствительность магнитно-резонансной томографии в рамках оценки динамики повреждений ПКС.

Ключевые слова: спортивная медицина; детские спортивные травмы; повреждения крупных суставов; PRP-терапия; несовершеннолетние спортсмены

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INTRODUCTION

The development of physical culture and sports, including youth sports, is a priority direction of Russian state policy.[1,2] According to the list of instructions of the President of Russia,[3] the Federal Medical and Biological Agency (FMBA) serves as the lead organization for medical support and biomedical supervision of athletes in Russian national teams. This role, combined with the country's strategic national priorities in scientific and technological development, drives the adoption of innovative technologies in sports medicine.[4]

In Russia, approximately 24.3 million children regularly engage in physical culture, with 3.2 million training in sports centers. Elite sports involve about 17,000 children. Sports-related injuries account for about 35.8% of all injuries among children (aged 5–17) [1]. Knee injuries dominate (approximately 46% of all major joint injuries), with anterior cruciate ligament (ACL) injuries remaining highly prevalent among professional athletes, children, and adolescents. These are severe conditions, comprising 15–24% of such injuries, while meniscus trauma occurs at a rate of 5.1 per 100,000 juniors [2, 3]. Upper limb injuries (shoulder, elbow joints) are common among athletes (including children) involved in overhead activities. In 75% of cases, these injuries result in temporary exclusion from sports [4], yet only 5% require surgical intervention [5].

The development of physical culture and sports in Russia has led to a growing number of professional athletes, increasing the incidence of sports-related injuries. This trend necessitates the adoption of novel therapeutic approaches, including regenerative medicine technologies such as the use of platelet-rich plasma (PRP).

PRP, as an orthobiological agent and a biological substance derived from the patient's own body, accelerates recovery and promotes regeneration, alleviates pain, and shortens the rehabilitation period. This

[1] Decree of the Government of the Russian Federation No. 3081-r dated 24.11.2020 "On the Approval of the Strategy for the Development of Physical Culture and Sports in the Russian Federation for the Period up to 2030". URL: https://docs.cntd.ru/document/566430492
[2] Resolution of the Government of the Russian Federation No. 1661 dated 30.09.2021 "On the Approval of the State Program of the Russian Federation 'Development of Physical Culture and Sports". URL: https://base.garant.ru/402891691/
[3] List of Instructions of the President of the Russian Federation following the meeting of the Presidential Council for the Development of Physical Culture and Sport dated 29.11.2024, No. Pr-2500. URL: http://www.kremlin.ru/acts/assignments/orders/75738
[4] Decree of the President of the Russian Federation No. 145 dated 28.02.2024, "On the Strategy for Scientific and Technological Development of the Russian Federation". URL: http://www.kremlin.ru/acts/bank/50358

is particularly critical in elite sports [6, 7]. Moreover, when performed according to guidelines, PRP therapy is a minimally invasive technique with a low complication rate [8]. While numerous publications address the use of PRP for joint diseases in adults with osteoarthritis and in older professional athletes [9, 10], studies evaluating the efficacy of PRP in underage athletes with injuries to large joints remain insufficient.

This work presents a clinical case of a professional athlete with a knee joint injury, assessing the functional and clinical outcomes of PRP therapy.

Preparation of Platelet-Rich Plasma (PRP)

Injuries and damage to various segments of the limbs are an unfortunate but integral part of elite sports. PRP has been successfully applied in such conditions as tennis elbow, golfers elbow, Achilles tendon injuries and plantar fasciitis, rotator cuff syndrome, adductor enthesopathy, jumpers knee, and runners knee [11].

PRP is human blood plasma with a concentration of platelets exceeding physiological levels. Platelets are anucleate cytoplasmic bodies formed through the fragmentation of megakaryocyte precursors. They circulate in the blood, expressing glycoproteins on their cell membranes, playing a key role in hemostasis and wound healing through the formation of fibrin clots [12].

A number of studies have shown that platelet counts in healthy individuals change throughout life. The normal platelet count for adults of both sexes ranges (150–400)×10^9/L. In children, platelet levels vary with age during growth and development. For example, in a one-month-old child, the platelet count is (208–352)×10^9/L, reaching about (198–340)×10^9/L[5] by age 11.

PRP is obtained by separating platelets from other blood components through the collection of a specific volume of the patient's blood followed by centrifugation. This process yields blood plasma with a high platelet concentration (exceeding the physiological baseline by 3–10 times). The final platelet concentration may vary depending on the system used [13, 14].

Currently, a substantial number of scientific studies are dedicated to randomized clinical trials on the use of PRP therapy for injuries and disorders of the musculoskeletal system [15, 16], including in professional athletes. However, Russia lacks a unified approved methodology for injections of PRP. To standardize the approach to PRP application for injuries and damage to large joints in junior athletes, two methods have been approved at the Federal Scientific and Clinical Center for Children and Adolescents: open and closed (Fig. 1).

When processed using a closed-loop system, platelets are not exposed to the external environment after blood collection. This system involves the use of a commercial kit, primarily in combination with additional centrifugation.

There are numerous (over 40) protocols and commercial systems for producing PRP; the relevant data are presented in Table.

The study by Jildeh et al. present a comparative analysis of PRP preparation methods using commercial systems. Technical specifications, cost, processing time, and centrifugation parameters of various commercially available PRP devices differ significantly. The physician independently determines the characteristics that best align with their PRP requirements [17].

CLINICAL CASE DESCRIPTION

Patient A, 16 years old (born 2007), a professional beach volleyball athlete, was under observation at the Traumatology Department of the Federal Scientific and Clinical Center for Children and Adolescents with the following diagnosis: S83.7 Injury of multiple structures of the knee joint. Partial tear of the anterior cruciate ligament (ACL) of the left knee joint. W09.3 Fall involving sports ground equipment during sports activities and competitions.

Medical History. The patient is the first child from a physiological pregnancy and spontaneous delivery. Birth weight was 3450 g, length 52 cm, Apgar score 8–9. Breastfeeding continued until 10 months. Psychomotor development during the first year was age-appropriate. Past infectious diseases: ARVI, chickenpox. Preventive vaccinations were administered according to the national immunization schedule. No prior surgical interventions.

History of Present Illness. In March 2025, during training, the patient sustained a left knee injury due to a fall. Given the trauma and severe pain, magnetic resonance imaging (MRI) of the injured joint was performed outpatient at the local clinic. The MRI findings indicated an isolated ACL injury of the left knee joint (Fig. 2A). Two days post-injury, the patient was examined by a trauma orthopedist at the Federal Scientific and Clinical Center for Children and Adolescents.

Status Localis. Moderately pronounced edema in the left knee joint; positive patellar ballottement sign; flexion limited to 90° due to pain. Provocative tests (Lachman test, anterior drawer test) could not be performed at this stage due to severe pain. Pain intensity assessed via the Visual Analog Scale (VAS) (0 = no pain, 10 = worst pain) was 5 points.

Based on the history, complaints, physical examination, and diagnostic results, the final diagnosis was "Isolated injury of the anterior cruciate ligament of the left knee joint."

Management and Treatment Strategy. To reduce pain and stimulate regeneration of damaged tissues, a conservative treatment strategy was selected. This included immobilization of the knee joint (tutor for 2 weeks), troxerutin gel (for 2 weeks) cold therapy

[5] Normal Platelet Count in Blood. URL: https://wer.ru/articles/norma-trombotsitov-v-krovi/ (access date: 27.05.2025)

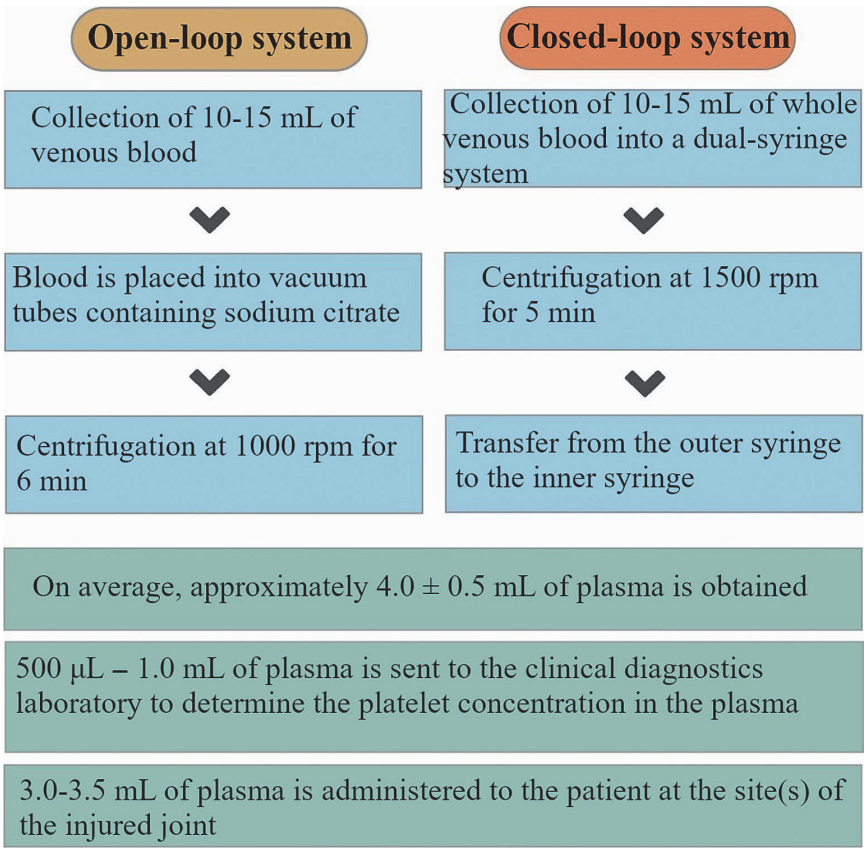


Figure adapted from [8]

Fig. 1. Methods of PRP application for injuries and damage to large joints in underage athletes

(3 days) for local treatment, of a hinged brace with gradual increase in flexion angle by 15–30° every 3 days, exclusion of running and jumping, and a course of PRP therapy (3 injections at 7-day intervals). After six months of the PRP therapy course, a follow-up MRI was conducted.

PRP Therapy. In accordance with the protocol developed and implemented at the Federal Scientific and Clinical Center for Children and Adolescents for candidate selection for PRP therapy, the patient was evaluated using inclusion criteria before initiating the PRP course. This included assessment of complete blood count (CBC) and inflammatory markers (C-reactive protein, CRP) to promptly identify signs of systemic inflammation or other contraindications. Additionally, prior to each injection, CBC was repeated to rule out potential contraindications (e.g., acute inflammatory processes, thrombocytopenia). Given normal laboratory values and no deviations from other inclusion criteria, the athlete received a course of PRP therapy consisting of three injections at seven-day intervals.

For platelet-rich plasma preparation, a closed-loop method using a dual-syringe system was selected: 15 mL of whole blood was collected from the medial cubital vein into Arthrex tubes and centrifuged at 1500 rpm for 5 min using a Rotofix 32A centrifuge. After blood component separation, the formed platelet-rich plasma layer (approximately 5.0 mL) was transferred from the outer syringe to the inner syringe. A portion of the prepared material (approximately 1.0 mL) was sent to a clinical diagnostic laboratory for platelet concentration measurement, which confirmed a twofold increase in platelet count compared to baseline. PRP injections were administered percutaneously under ultrasound guidance. The procedure was performed under aseptic conditions after skin antisepsis, with the patient in a supine position and the knee flexed at 90°. The injection was delivered into the knee joint cavity via a lateral approach. The patient tolerated PRP therapy satisfactorily, with no adverse reactions reported.

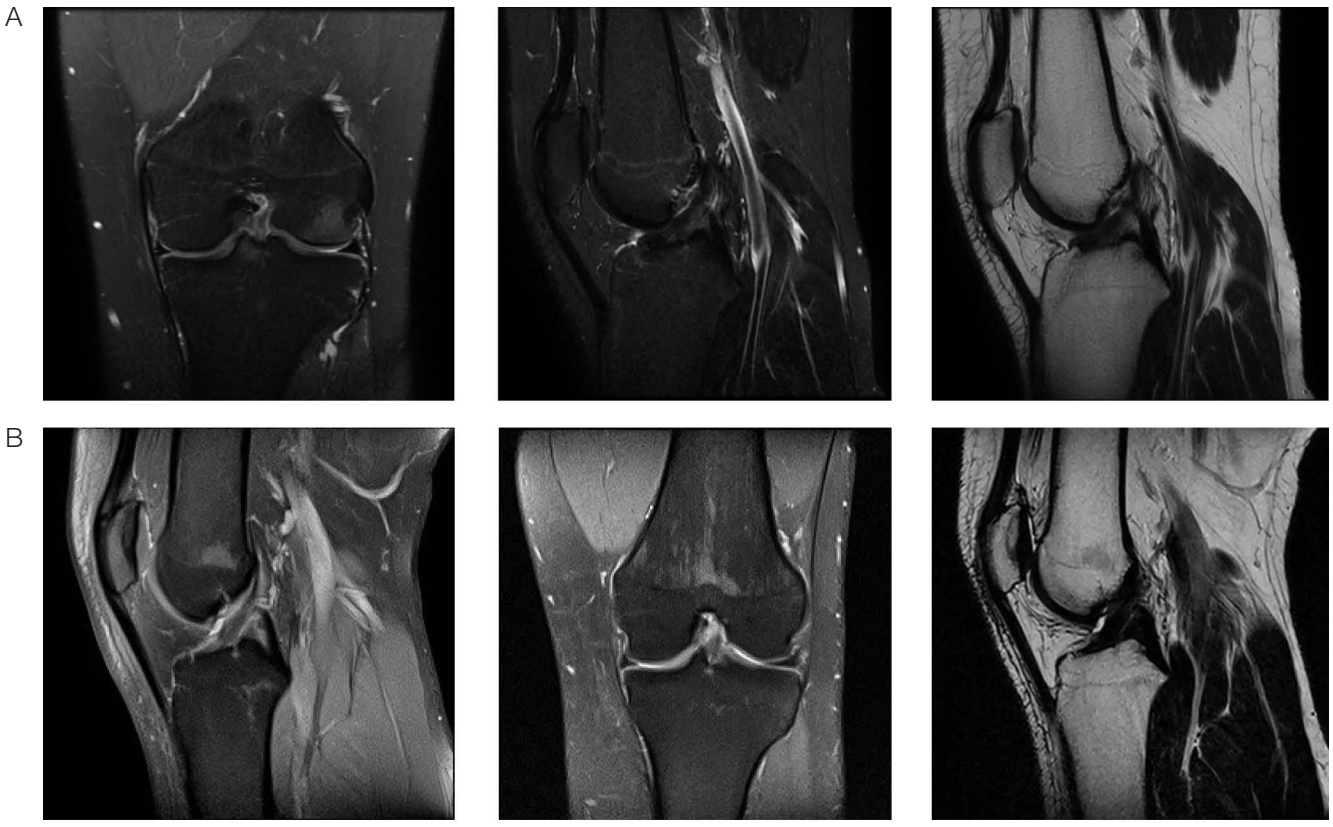
Therapy Results. After the completed course of PRP therapy, the patient noted improvement in knee joint function manifested in reduced pain syndrome (VAS – 3 points), improved flexion function, and decreased discomfort phenomena in the left knee joint during physical exercises.

The follow-up examination after six weeks showed a complete regression of the edema of the knee joint area, a full restoration of the joint flexion, and the absence of pain syndrome (VAS – 1 point). Slight limping was noted when leaning on the injured limb. Provocative tests (Lachman test and anterior drawer test) were questionable; however, no signs of pronounced instability were detected. Importantly for the athlete, gradual return to

Table. Point-of-Care processing methods for platelet-rich plasma

Name of Commercial Kit	Claimed Platelet Increase Fold (Times)	Platelet Increase Fold (Times)	Centrifugation Time (min)	Key Advantages	Disadvantages
ProofPoint	No data available	5.2	49	More than 4-fold increase in platelet count in plasma	Transfer via syringe. open system; 2 centrifugation stages
AcCELLerated	4	5.2	18	More than 4-fold increase in platelet count in plasma	Absent
Arthrex	2–3	4.2	15	Reduction of processing time; More than 4-fold increase in platelet count in plasma	Non-permanent increase in platelet count
Celling	No data available	2.7	29	Usability	Non-permanent increase in platelet count
Terumo	3.62	4.1	24	More than 4-fold increase in platelet count in plasma	Variability of results

Table prepared by the authors using own data [17]



MRI image obtained by the authors.

Fig. 2. Magnetic resonance imaging of the left knee joint: A — after injury; B — after treatment

previous sports load was noted, including running and cycling. Five months after the injury, the patient was able to resume gym training (leg press with 120 kg weight).

The follow-up examination six months after the injury showed the ability of the patient to walk independently and confidently, without limping. No edema in the left knee joint area was present; the patella was in the midline. Ballottement sign was negative, Lachman and anterior drawer tests were negative, symmetrical on both sides. No subjective or objective signs of knee joint instability were detected, VAS was 0 points. Therefore, according to control MRI data, restoration of the anterior cruciate ligament structure was confirmed (Fig. 2B).

Thus, this clinical case demonstrates the efficacy, safety, and good tolerability of PRP application for ACL injury in an underage professional athlete. Positive outcomes were observed both clinically — manifested as regression of pain assessed via VAS, restoration of joint function, and positive dynamics in provocative tests (Lachman test and anterior drawer test) — and through MRI data showing restoration of the anatomical integrity of the ligament. This highlights the importance of an individualized approach to treatment strategies for patients, particularly athletes with injuries and damage to large joints. The results align with current literature data, indicating the promise of therapeutic approaches in orthopedics and sports medicine utilizing regenerative medicine technologies.

CLINICAL CASE DISCUSSION

Anterior cruciate ligament (ACL) injury remains a common and severe trauma among professional athletes and sports enthusiasts [2]. Due to the high involvement of the ACL in knee mobility and stability, its injuries are of particular interest to sports medicine specialists, necessitating reduced rehabilitation time, lower probability of long-term complications, and faster recovery and return of athletes to elite sports. In this context, regenerative medicine technologies, including the use of platelet-rich plasma (PRP), hold a special place in orthopedics. Numerous research articles on PRP therapy have been published, demonstrating positive outcomes such as pain relief, stimulation of tissue regeneration, and accelerated healing processes in patients with musculoskeletal injuries [18]. PRP therapy is a minimally invasive method that can be used both as a monotherapy and in combination with conservative treatment. To accurately assess the effectiveness of PRP, comprehensive and dynamic clinical and instrumental examination is required. Thorough history-taking, pain assessment, and physical examination are helpful in determining the most appropriate diagnostic tools. Some studies prioritize magnetic resonance imaging (MRI) for diagnosing ligamentous injuries of the knee joint in children [19].

Indeed, MRI is widely used for evaluating knee injuries, but its accuracy in cases of damage to various structures of the knee joint remains uncertain. However, for ACL injuries, the high sensitivity of this method amounting to 90.4% has been proven [20].

In the presented clinical case, the use of PRP for ACL injury in an underage professional athlete showed favorable results both in terms of pain relief and restoration of functional abilities of the injured limb. It is important to note that this method is safe when procedural requirements are met (asepsis, antisepsis, ultrasound guidance, etc.), cost-effective due to the use of the patient's own plasma, and yields rapid results. The use of a closed-loop system for PRP preparation minimized potential risks, reduced the consumption of medical supplies, and shortened the time required for transferring PRP between tubes, thereby enhancing economic efficiency.

CONCLUSION

The study presents standardized methodologies for PRP therapy in injuries and damage to large joints in underage athletes. The efficacy of PRP application for ACL injury in a professional beach volleyball athlete was evaluated. A set of trigger points to track dynamic changes during PRP therapy for ACL injuries was theoretically substantiated, including clinical presentation with pain assessment via VAS, physical examination data, results of specific functional tests (Lachman test and anterior drawer test), and MRI diagnostics. The presented clinical case demonstrates the effectiveness of PRP therapy, with positive dynamics on radiological diagnostic results, culminating in complete restoration of the anterior cruciate ligament structure within the period of six months.

Given the existing domestic and international experience of using PRP for diseases and injuries of large joints in older population cohorts, as well as the results obtained in this clinical case, it appears promising to continue the application of PRP in underage athletes with injuries and damage to large joints. Subsequent evaluation of the efficacy of the proposed method should consider injury localization, type of damaged tissue, time since injury, and rehabilitation period, with documentation of clinical and functional dynamics.

References

1. Rjabov VP, Nurullin IF, Kurmaev ZF, Sungatullin RI, Belov AM *Professional injuries and diseases in athletes*. Kazan: Kazan Federal University; 2017 (In Russ.).
2. Campbell CJ, Carson JD, Diaconescu ED, Celebrini R, Rizzardo MR, Godbout V, et al. Canadian Academy of Sport and Exercise Medicine position statement: Neuromuscular training programs can decrease anterior cruciate ligament injuries in youth soccer players. *Clinical Journal of Sport Medicine.* 2014;24:263–7. https://doi.org/10.1097/jsm.0000000000000068
3. Mitchell J, Graham W, Best TM, Collins C, Currie DW, Comstock RD, et al. Epidemiology of meniscal injuries in US high school athletes between 2007 and 2013. *Knee Surgery, Sports Traumatology, Arthroscopy.* 2016;24:715–22. https://doi.org/10.1007/s00167-015-3814-2
4. McFarland EG, Wasik M. Epidemiology of collegiate baseball injuries. *Clinical Journal of Sport Medicine.* 1998;8(1):10–3. https://doi.org/10.1097/00042752-199801000-00003
5. Fleisig GS, Andrews JR, Cutter GR, Weber A, Loftice J, McMichael C, et al. Risk of serious injury for young baseball pitchers: a 10-year prospective study. *The American Journal of Sports Medicine.* 2011;39:253–7. https://doi.org/10.1177/0363546510384224
6. Bray CC, Walker CM, Spence DD. Orthobiologics in pediatric sports medicine. *Orthopedic Clinics of North America*. 2017;48(3):333–42. https://doi.org/10.1016/j.ocl.2017.03.006
7. Godek P. High-volume PRP therapy. *Ortopedia Traumatologia Rehabilitacja.* 2022;24(1):43–60. https://doi.org/10.5604/01.3001.0015.7806
8. Malanin DA, Tregubov AS, Demeshenko MV, Cherezov LL. *PRP-therapy for osteoarthritis of large joints.* Volgograd: VolgGMU Publishing House; 2018 (In Russ.).
9. Xiong Y, Gong C, Peng X, Liu X, Su X, Tao X, et al. Efficacy and safety of platelet-rich plasma injections for the treatment of osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. *Frontiers in Medicine*. 2023;10:1204144. https://doi.org/10.3389/fmed.2023.1204144
10. Pontes de Macedo A, Santos Duarte Lana JF, Pedrozo CM, Corrêa Bottene I, De Medeiros JRM, Da Silva LQ. The regenerative medicine potential of PRP in elite athlete injuries. *Fortune Journal of Rheumatology.* 2020;2:16–26. https://doi.org/10.26502/fjr.26880014
11. Ficek K, Kamiński T, Wach E, Cholewiński J, Cięszczyk P Application of platelet-rich plasma in sports medicine. *Journal of Human Kinetics.* 2011;30:85–97.
12. Herdea A, Struta A, Derihaci R, Ulici A, Costache A, Furtunescu F, et al. Efficiency of platelet-rich plasma therapy for healing sports injuries in young athletes. *Experimental and Therapeutic Medicine.* 2022;23(3):1–6. https://doi.org/10.3892/etm.2022.11139
13. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA Platelet-rich plasma from basic science to clinical applications. *The American Journal of Sports Medicine*. 2009;37(11):2259–72. https://doi.org/10.1177/0363546509349921
14. Medina-Porqueres I, Martin-Garcia P, Sanz-De-Diego S, Gomez-Caceres A, Moya-Torrecilla F, Reyes-Eldblom M, et al. Clinical and functional outcome of meniscal injuries treated with platelet-rich plasma: a single-center case series. *International Journal of Environmental Research and Public Health.* 2022;19(12):7118. https://doi.org/10.3390/ijerph19127118
15. Zayni R, Thaunat M, Fayard JM, Hager JP, Carrillon Y, Clechet J, et al. Platelet-rich plasma as a treatment for chronic patellar tendinopathy: comparison of a single versus two consecutive injections. *Muscles, Ligaments Tendons Journal.* 2015;5(2):92–8.
16. Charousset C, Zaoui A, Bellaiche L, Bouyer B. Are multiple platelet-rich plasma injections useful for treatment of chronic patellar tendinopathy in athletes? A prospective study. *The American Journal of Sports Medicine*. 2014;42(4):906–11. https://doi.org/10.1177/0363546513519964
17. Jildeh TR, Su CA, Vopat ML, Brown JR, Huard J. A review of commercially available point-of-care devices to concentrate platelet-rich plasma. *Cureus*. 2022;14(8):e28498. https://doi.org/10.7759/cureus.28498
18. Zhang JY, Fabricant PD, Ishmael CR, Wang JC, Petrigliano FA, Jones KJ. Utilization of platelet-rich plasma for musculoskeletal injuries: an analysis of current treatment trends in the United States. *Orthopaedic Journal of Sports Medicine.* 2016;4(12):2325967116676241. https://doi.org/10.1177/2325967116676241
19. Askenberger M, Arendt EA, Ekström W, Voss U, Finnbogason T, Janarv PM. Medial patellofemoral ligament injuries in children with first-time lateral patellar dislocations: a magnetic resonance imaging and arthroscopic study. *The American Journal of Sports Medicine*. 2016;44(1):152–8. https://doi.org/10.1177/0363546515611661
20. Li X, Hou Q, Zhan X, Chang L, Ma X, Yuan H. The accuracy of MRI in diagnosing and classifying acute traumatic multiple ligament knee injuries. *BMC Musculoskeletal Disorders.* 2022;23:43. https://doi.org/10.1186/s12891-021-04976-1

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INFLUENCE OF DENTAL AND ORTHODONTIC DISEASES ON PHYSICAL PERFORMANCE AND ENDURANCE OF HIGH-CLASS COMBAT SPORTS ATHLETES

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Introduction. Oral health issues, such as dental caries, periodontal diseases, or malocclusion, can cause pain, discomfort, and systemic health problems, which in turn may negatively affect the performance and endurance of athletes. In this context, the development of comprehensive dental programs for athletes involved in professional sports is a relevant task.
Objective. To determine statistically significant differences in performance parameters among combat sports athletes for the development of measures to correct the dental status of highly qualified athletes.
Materials and methods. A mathematical and statistical analysis of anonymized medical data on comprehensive medical examination of elite athletes was conducted. Data from 1887 combat sports athletes were processed (n = 1887; males n = 1190; females n = 697). The sample was divided into two groups: athletes without dental pathologies — Group 0 (n = 791; M_e median age 21.00 [19.00; 25.00]); athletes with confirmed dental pathologies — Group 1 (n = 1096; M_e median age 19.00 [17.00; 24.00]). Diagnoses from endocrinologists and gastroenterologists were also taken into account. Morphometric characteristics and physiological parameters from exercise stress testing were analyzed. Statistical analysis was performed using the StatTech v. 4.6.0 software.
Results. A significant influence of dental diseases on physical performance and endurance was identified. Compared to the group of athletes without a dental diagnosis (Group 0), the presence of a dental diagnosis (Group 1) was associated with statistically significant differences ($p < 0.05$) across a range of physiological indicators characterizing physical endurance and performance: respiratory exchange ratio $R(0)$ = 1.05 [1.03; 1.09], $R(1)$ = 1.04 [1.03; 1.07]; heart rate at the aerobic threshold level $HR_{AerT}(0)$ = 110.00 [100.00; 122.00], $HR_{AerT}(1)$ = 114.00 [102.00; 126.00]; heart rate at the anaerobic threshold $HR_{AT}(0)$ = 143.00 [132.00; 154.00], $HR_{AT}(1)$ = 147.00 [134.00; 158.00]; peak heart rate at peak load $HR_{peak}(0)$ = 151.00 [144.00; 160.00], $HR_{peak}(1)$ = 152.00 [144.00; 163.00]; heart rate at the 3rd min of recovery $HR_{3min}(0)$ = 91.00 [82.00; 101.00], $HR_{3min}(1)$ = 93.00 [84.00; 102.00]; power output at the level of the anaerobic threshold $Pwr_{AT}(0)$ = 190.00 [165.00; 230.00], $Pwr_{AT}(1)$ = 200.00 [165.00; 240.00].
Conclusions. Dental diseases reduce the performance athletes, in particular at submaximal load levels. This has a negative effect on the training process and competitive results in martial arts. In this regard, a comprehensive prevention program and regular dental checkups are recommended as an essential part of preparation, in particular, in contact sports. The use of individual aligners for mitigating excessive impact on teeth under the conditions of overload and extreme situations is proposed.

Keywords: dentistry; sports medicine; combat sports athletes; comprehensive medical examination; aligners; retrospective study; physical performance; physical endurance

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ВЛИЯНИЕ СТОМАТОЛОГИЧЕСКИХ И ОРТОДОНТИЧЕСКИХ ЗАБОЛЕВАНИЙ НА ФИЗИЧЕСКУЮ РАБОТОСПОСОБНОСТЬ И ВЫНОСЛИВОСТЬ СПОРТСМЕНОВ-ЕДИНОБОРЦЕВ ВЫСОКОГО КЛАССА

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Введение. Проблемы со здоровьем полости рта, такие как кариес зубов, заболевания пародонта и неправильный прикус, могут вызывать боль, дискомфорт и системные проблемы со здоровьем, что, в свою очередь, может негативно сказаться на работоспособности и выносливости спортсмена. Наряду с этим существует и необходимость разработки комплексных стоматологических программ для профессионального спорта.

Цель. Определение статистически значимых различий параметров работоспособности у спортсменов-единоборцев для разработки мероприятий по коррекции стоматологического статуса у высококвалифицированных спортсменов.

Материалы и методы. Проведен математико-статистический анализ деперсонализированных медицинских данных результатов углубленного медицинского обследования спортсменов высокого класса. Обработаны данные 1887 представителей спортивных единоборств (n = 1887; мужчины n = 1190; женщины n = 697). Выборка была разделена на 2 группы: спортсмены без стоматологической патологии — группа «0» (n = 791; M_e среднего возраста 21,00 [19,00; 25,00]); спортсмены со стоматологическими диагнозами — группа «1» (n = 1096; M_e среднего возраста 19,00 [17,00; 24,00]). В работе учитывали также диагнозы эндокринолога и гастроэнтеролога. Анализировали морфометрические характеристики и физиологические показатели нагрузочного тестирования. Статистический анализ проводился с использованием программы StatTech v. 4.6.0.

Результаты. Выявлено значительное влияние стоматологических заболеваний на физическую работоспособность и выносливость. Наличие стоматологического диагноза (группа «1») связано со статистически значимыми различиями (p < 0,05) по сравнению с группой спортсменов без стоматологического диагноза (группа «0»), по ряду физиологических показателей характеризующих физическую выносливость и работоспособность: дыхательный коэффициент $R(0)$ = 1,05 [1,03; 1,09], $R(1)$ = 1,04 [1,03; 1,07]; частота сердечных сокращений аэробного порога $ЧСС_{АП}(0)$ = 110,00 [100,00; 122,00], $ЧСС_{АП}(1)$ = 114,00 [102,00; 126,00]; частота сердечных сокращений на уровне анаэробного порога $ЧСС_{ПАНО}(0)$ = 143,00 [132,00; 154,00], $ЧСС_{ПАНО}(1)$ = 147,00 [134,00; 158,00]; частота сердечных сокращений на пике нагрузки $ЧСС_{ПИК}(0)$ =151,00 [144,00; 160,00], $ЧСС_{ПИК}(1)$ = 152,00 [144,00; 163,00]; частота сердечных сокращений на 3-й минуте восстановления $ЧСС_{3\ мин}(0)$ = 91,00 [82,00; 101,00], $ЧСС_{3\ мин}(1)$ = 93,00 [84,00; 102,00]; мощность ступени, на которой достигнут уровень порога анаэробного обмена, $Мощ_{ПАНО}(0)$ = 190,00 [165,00; 230,00], $Мощ_{ПАНО}(1)$ = 200,00 [165,00; 240,00].

Выводы. Стоматологические заболевания снижают работоспособность спортсменов, особенно на субмаксимальных уровнях нагрузки, что может негативно сказываться на тренировочном процессе и соревновательных результатах в боевых искусствах. В связи с этим рекомендованы комплексная программа профилактики и регулярные стоматологические осмотры как обязательная часть подготовки, особенно в контактных видах спорта. На основании анализа результатов исследования предложено использовать индивидуальные элайнеры для предотвращения избыточного воздействия на зубы в условиях перегрузок и экстремальных ситуаций.

Ключевые слова: стоматология; спортивная медицина; единоборцы; углубленное медицинское обследование; элайнеры; ретроспективное исследование; физическая работоспособность; физическая выносливость

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INTRODUCTION

Dental and orthodontic diseases represent a multifaceted problem that directly or indirectly affects the performance of athletes. Oral diseases can cause severe pain, which directly impacts the ability of athletes to train and compete [1]. This is particularly relevant in combat sports, where physical conditioning, concentration, and endurance are crucial. The presence of oral diseases can affect the ability to eat properly, leading to nutritional deficiencies that negatively impact physical performance and endurance. Pain caused by dental caries or periodontal disease can result in reduced training volumes and complicate participation in competitions. Teixeira et al. demonstrated that athletes with oral diseases may experience up to a 21% decline in performance [2]. Unsatisfactory dental conditions can lead to systemic diseases, such as cardiovascular and respiratory complications, impairing muscle recovery and overall physical performance [2]; negatively affect the confidence and quality of life of athletes [3], their appearance and social interactions, which are extremely important for maintaining a positive mental state [4]. Additionally, there is evidence suggesting that oral health problems can impact cognitive functions necessary for strategic thinking and decision making [5].

This study aims to determine statistically significant differences in performance parameters among combat sports athletes to develop measures for correcting the dental status of highly qualified athletes.

MATERIALS AND METHODS

A mathematical and statistical analysis of anonymized medical data from the comprehensive medical examination (CME) of high-class athletes, not lower than Masters of Sport of Russia, was conducted.

Data on 1887 (n = 1887; males n = 1190; females n = 697) representatives of combat sports, including aikido, arm wrestling, boxing, wrestling, belt wrestling,

freestyle wrestling, Greco-Roman wrestling, grappling, jiu-jitsu, judo, karate, kickboxing, Kyokushin, hand-to-hand fighting sport, savate, sambo, sumo, Muay Thai, taekwondo, universal fighting, and wushu, were analyzed. The sample was divided into two groups: athletes without dental pathologies (median age 21.00 [19.00; 25.00]); athletes with dental diagnoses (median age 19.00 [17.00; 24.00]). Athletes with a dental diagnosis listed under K02–K08.9 according to ICD-10 formed Group 1 (n = 1096); those without a dental diagnosis (n = 791) formed Group 0.

The study also considered endocrinological (ICD-10 codes: E00–E07, E10–E16, E40–E46, E65–E68, E70–E90) and gastroenterological (ICD-10 codes: K00–K93) diagnoses.

Data for both groups were compared based on the results of the CME, including functional testing on a bicycle ergometer using the Ramp-30 protocol. This is a method of stress testing involving ergospirometry on a V-ergoPro bicycle ergometer: "to failure" under gradually increasing load starting from 5 W.

The inclusion criteria for the study were completion of the CME and the athlete's clearance for competitions.

For subsequent processing, the following quantitative indicators were identified: height (cm); weight (kg); oxygen consumption at the anaerobic threshold level ($VO_{2\ AT}$, mL/min/kg), i.e., the amount of oxygen utilized primarily by working muscles per unit time at the moment of reaching the anaerobic threshold; oxygen consumption at the maximum stage of load testing ($VO_{2\ peak}$, mL/min/kg), i.e., the amount of oxygen utilized primarily by working muscles per unit time at the maximum achieved power during testing with submaximal loads (if $VO_{2\ max}$ — maximal oxygen consumption — is not reached, then it coincides with $VO_{2\ max}$); respiratory exchange ratio (R, relative units), i.e., the ratio of carbon dioxide released to oxygen consumption, reflecting the ratio of oxidized substrates, ventilation-perfusion ratios in the lungs, and the activity of the blood bicarbonate buffer; heart rate before exercise (HR_{rest}, bpm), which depends on age, gender, stage of the training process, and skill level; heart rate at the aerobic threshold (HR_{AerT}, bpm), which is the upper limit of the individual aerobic zone and the lower limit for the developmental zone of exercise intensity; heart rate at the anaerobic threshold level (HR_{AT}, bpm), which is the upper limit of the developmental and lower limit of the anaerobic individual zones of exercise intensity; heart rate at peak load (HR_{peak}, bpm), i.e., the maximum recorded heart rate during exercise "to failure"; heart rate at the 3rd min of recovery ($HR_{3\ min}$, bpm), i.e., supporting the body's recovery processes, one of the criteria for assessing fitness; power output at the level of the anaerobic threshold (Pwr_{AT}, W), reflecting the absolute power that an athlete can generate at the anaerobic threshold level; power output at the maximum stage of testing (Pwr_{peak}, W), reflecting the absolute power that an athlete can generate; relative power at the anaerobic threshold level (Pwr_{AT}/weight, W/kg), i.e., a relative indicator that takes into account the athlete's body weight and allows for comparing work efficiency considering individual physical parameters (it is particularly important when assessing athletes of different weights, due to its more accurate representation of functional capabilities per unit of body weight); (Pwr_{peak}/weight, W/kg) is the relative power at peak load.

In modeling formulas, quantitative indicators are represented by adding the index X to the studied parameters and Y to the analyzed value; diagnoses from gastroenterologists, dentists, and endocrinologists (presence of diagnosis — 1, absence of diagnosis — 0); gender (0 — female (F), 1 — male (M)).

Quantitative indicators were assessed for compliance with normal distribution using the Shapiro–Wilk test (for sample sizes less than 50) or the Kolmogorov–Smirnov test (for sample sizes greater than 50). Quantitative indicators with normal distribution were described using arithmetic means (M) and standard deviations (SD), and 95% confidence interval (95% CI) limits. In the absence of normal distribution, quantitative data were described using median (M_e) and lower and upper quartiles [Q_1; Q_3] ([IQR]). Comparison of the study groups for a quantitative indicator with normal distribution, assuming equal variances, was performed using Student's t-test; for unequal variances, Welch's t-test was used. Comparison of the study groups for a quantitative indicator with a distribution different from normal was performed using the Mann–Whitney U-test.

A predictive model characterizing the dependence of the quantitative variable HR_{AerT} (heart rate at aerobic threshold) on gender, diagnoses from gastroenterologists, dentists, endocrinologists, weight, and indicators HR_{rest}, HR_{AT}, HR_{3min}, Pwr_{AT}, Pwr_{AT}/weight, was developed using the linear regression method. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using the StatTech v. 4.6.0 software (StatTech, Russia).

RESULTS AND DISCUSSION

Table 1 presents the results of descriptive statistics for groups of high-class combat athletes.

Table 1 demonstrates statistically significant differences between the groups of athletes with (Group 1) and without a dental diagnosis (Group 0) across several parameters. For instance, heart rate before exercise was higher in Group 1 (M_e = 81.00 bpm) compared to Group 0 (M_e = 78.00 bpm, $p < 0.001$). A similar trend was observed for heart rate at the aerobic threshold (HR_{AerT}) (114.00 vs. 110.00 bpm, $p < 0.001$) and heart rate at the anaerobic threshold (HR_{AT}) (147.00 vs. 143.00 bpm, $p < 0.001$). These differences may indicate higher baseline activation of the sympathetic nervous system in athletes with oral health issues, likely associated with chronic inflammation or pain caused by dental caries or periodontitis. Studies by other authors confirm that inflammatory processes in the oral cavity can exacerbate

Table 1. Descriptive statistics of quantitative variables by presence of dental diagnosis

Parameters	Dental report		Statistical significance level, *p*
	Group 0 (*n* = 791) M_e [IQR]	Group 1 (*n* = 1096) M_e [IQR]	
Age, years	21.00 [19.00; 25.00]	19.00 [17.00; 24.00]	0.285
Height, cm	174.00 [167.00; 182.00]	173.00 [166.00; 181.00]	0.285
Body weight, kg	73.00 [63.00; 87.00]	73.00 [63.00; 87.00]	0.852
Oxygen consumption at the anaerobic threshold level ($VO_{2\ AT}$), mL/min/kg	31.02 [26.68; 35.45]	31.31 [26.84; 36.11]	0.333
Oxygen consumption at the maximum stage of exercise testing ($VO_{2\ peak}$), mL/min/kg	33.84 [29.59; 38.18]	33.59 [29.01; 38.03]	0.449
Respiratory exchange ratio (*R*), rel. units	1.05 [1.03; 1.09]	1.04 [1.03; 1.07]	0.002
Heart rate before exercise (HR_{rest}), beats per min (bpm)	78.00 [70.00; 87.00]	81.00 [72.00; 89.00]	<0.001
Heart rate at aerobic threshold (HR_{AerT}), beats per min (bpm)	110.00 [100.00; 122.00]	114.00 [102.00; 126.00]	<0.001
Heart rate at anaerobic threshold (HR_{AT}), beats per min (bpm)	143.00 [132.00; 154.00]	147.00 [134.00; 158.00]	<0.001
Heart rate at peak load (HR_{peak}), beats per min (bpm)	151.00 [144.00; 160.00]	152.00 [144.00; 163.00]	0.025
Heart rate at 3 min of recovery (HR_{3min}), beats per min (bpm)	91.00 [82.00; 101.00]	93.00 [84.00; 102.00]	0.017
Power output at the anaerobic threshold level (Pwr_{AT}), W	190.00 [165.00; 230.00]	200.00 [165.00; 240.00]	0.028
Power output at the maximum stage of testing (Pwr_{Peak}), W	215.00 [180.00; 260.00]	215.00 [175.00; 260.00]	0.982
Relative power output at anaerobic threshold per body weight (Pwr_{AT}/weight), W/kg	2.63 [2.27; 3.01]	2.72 [2.29; 3.16]	0.006
Relative maximal power output per body weight during testing (Pwr_{Peak}/weight), W/kg	2.94 [2.59; 3.31]	2.96 [2.54; 3.36]	0.568

Table compiled by the authors based on their own data

Note: IQR — interquartile range

systemic stress, increasing cortisol levels and affecting cardiovascular regulation [12].

Furthermore, power output at the anaerobic threshold (W) and relative power at the anaerobic threshold (W/kg) were higher in Group 1 ($p = 0.028$ and $p = 0.006$, respectively), which may suggest compensatory mechanisms. Thus, athletes with chronic pain or discomfort might exert more effort to achieve the same performance level. However, peak power and $VO_{2\ peak}$ showed no significant differences ($p = 0.982$ and $p = 0.449$), indicating that dental issues have a limited impact on maximal aerobic capacity.

For each parameter reflecting the performance and endurance of athletes, regression models were developed. Our approach was strictly guided by statistical analysis, and we present robust mathematical models. The strength and closeness of the relationship between the studied parameters were assessed based on the correlation coefficient, considering the appropriate level of statistical significance and the observed variance

magnitude. The corresponding data are presented in Table 2.

The observed dependence of the HR_{AerT} (bpm) indicator is described by the following linear regression equation:

$$Y_{HR\ AerT} = -18.046 - 4.633 \times X_{M} + 5.500 \times X_{gastro} + 1.375 \times X_{dent} + 2.219 \times X_{endo} + 0.167 \times X_{weight} + 0.120 \times X_{HR\ rest} + 0.646 \times X_{HR\ AT} + 0.130 \times X_{HR\ 3min} - 0.079 \times X_{PWR\ AT} + 8.007 \times X_{PWR\ AT/weight}.$$

Based on the presented regression model equation, athletes can expect an increase in HR_{AerT} with a gastroenterological diagnosis (by 5.500 bpm), a dental diagnosis (by 1.375 bpm), and an endocrinological diagnosis (by 2.219 bpm).

The HR_{AerT} indicator will increase with a 1 bpm increase in HR_{rest} — by 0.120 bpm; with each kilogram increase in the athlete's body weight — by 0.167 bpm; with a 1 bpm increase in HR_{AT} — by 0.646 bpm; with a 1 bpm increase in HR_{3min} — by 0.130 bpm; with a 1 W/kg increase in Pwr_{AT}/weight — by 8.007 bpm. At the same time, with an increase in Pwr_{AT}, a decrease in the HR_{AerT} indicator by 0.079 bpm can be expected. According to the regression model results, males are expected to have a decrease in HR_{AerT} by 4.633 bpm compared to females.

The obtained regression model is characterized by a multiple correlation coefficient R_{xy} = 0.830; ($p < 0.001$), which corresponds to a high strength of association according to the Chaddock scale. The obtained model allowed the change in the HR_{AerT} indicator to be predicted with high accuracy, accounting for 68.9% of the observed variance. The assessment of the dependence of HR_{AT} (bpm) on quantitative factors was performed using the linear regression method. The number of observations was 1887.

The linear regression results (Table 2) confirm that the presence of a dental diagnosis is independently associated with an increase in HR_{AerT} by 1.375 bpm ($p = 0.017$). Although this effect seems minor, it has cumulative significance in the context of other factors, such as gender (increase in HR_{AerT} in men by 4.633 bpm, $p < 0.001$), presence of a gastroenterological diagnosis (increase by 5.500 bpm, $p = 0.021$), and endocrinological problems (increase by 2.219 bpm, $p = 0.039$). The model explains 68.9% of the variance in HR_{AerT} ($R_{xy} = 0.830$, $p < 0.001$), indicating high predictive power and confirming the systemic nature of the influence of dental diseases.

Interestingly, an increase in power at the anaerobic threshold (Pwr_{AT}) is associated with a decrease in HR_{AerT} (-0.079 bpm per 1 W, $p = 0.007$), which may reflect better cardiovascular adaptation to exercise in more trained

Table 2. Statistical description of the regression model

Parameters	Contribution in regression equation	Standard error of the mean	*t*-test of regression model parameters	Statistical significance level, *p*
Intercept	-18.046	6.357	2.839	0.005
Gender: M	4.633	0.759	6.105	<0.001
Gastroenterologist	5.500	2.389	2.302	0.021
Dentist	1.375	0.573	2.398	0.017
Endocrinologist	2.219	1.073	2.069	0.039
Body weight, kg	0.167	0.074	2.262	0.024
Heart rate before exercise (HR_{rest}), beats per min (bpm)	0.120	0.029	4.174	<0.001
Heart rate at anaerobic threshold (HR_{AT}), beats per min (bpm)	0.646	0.027	23.870	<0.001
Heart rate at 3 min of recovery (HR_{3min}), beats per min (bpm)	0.130	0.024	5.470	<0.001
Power output at the anaerobic threshold level (Pwr_{AT}), W	-0.079	0.029	-2.706	0.007
Relative power output at anaerobic threshold per body weight (Pwr_{AT}/weight), W/kg	8.007	2.340	3.421	< 0.001

Table compiled by the authors based on their own data

Note: *Intercept* — dimensionless indicator

athletes. However, an increase in relative power at the anaerobic threshold (Pwr_{AT}/weight) increases HR_{AerT} by 8.007 bpm ($p < 0.001$), highlighting the complex relationship between body weight, strength, and cardiac response. It should be noted that, despite the significance of the identified differences in cardiorespiratory parameters, peak power output metrics showed no statistically significant differences between the groups. This may indicate that short-term maximal performance remains unaffected, while endurance and recovery speed are impaired, which is particularly critical for combat sports athletes.

The data obtained are consistent with those reported in literature demonstrating that oral health affects athletic performance through several mechanisms. First, chronic pain from dental caries or periodontitis can reduce training volumes and concentration, which is particularly critical in martial arts where strategic thinking is required [13]. Second, systemic inflammation caused by periodontal infections may impair muscle recovery and increase the risk of cardiovascular complications [14]. Third, nutritional problems due to pain or tooth loss can lead to deficiencies in macronutrients and micronutrients, thus reducing endurance [15].

Our study used the data of comprehensive medical examinations (CME) of 1887 combat sports athletes to reveal a significant impact of dental diseases on physical performance and endurance. The analysis showed that the presence of dental problems leads to changes in a range of physiological indicators, including heart rate (HR) during various phases of exercise and recovery, as well as power output at the anaerobic threshold (AT). These results underscore the importance of oral health as a factor influencing athletic performance [5], particularly in martial arts, which demand a high level of physical conditioning [8] and concentration [1, 9].

The identified dependence of HR_{AT} and HR_{peak} on the presence of dental diseases confirms the hypothesis of the negative impact of dental pathologies on the adaptive capabilities of athletes. Elevated HR during various exercise phases may indicate reduced efficiency of energy metabolism and slower recovery processes, which are crucial in professional sports. The conducted regression analysis also revealed that, in addition to dental diseases, physiological parameters of athletes are influenced by conditions affecting other body systems, such as gastrointestinal and endocrine pathologies [10, 11]. This reinforces the need for comprehensive medical support for athletes, with an emphasis on an interdisciplinary approach.

The obtained data are consistent with the results of similar studies [16, 17] and indicate a high prevalence of dental diseases affecting physiological indicators and athletic performance among combat sports athletes. The presence of dental pathologies is associated with increased load on the cardiovascular system [19], which may reduce the body's adaptive capabilities to physical exertion and impair recovery processes [18, 20–22]. Many athletes lack sufficient awareness and, consequently, adherence to preventive oral hygiene practices [7], leading to the neglect and exacerbation of oral health problems [5]. Increasing awareness among athletes, coaches, and sports organizations about the importance of oral hygiene could lead to improved preventive measures and better outcomes [10]. The use of aligners or braces for bite correction, adapted for contact sports [23], may serve as an effective prevention measure of orthodontic issues. Regular dental checkups and the use of protective devices, such as custom-made mouthguards, can help prevent oral injuries and diseases [1]. Effective strategies for promoting oral health are necessary to minimize its impact on performance [4].

The similarity of psychophysiological stress during combat in martial artists to the overload states experienced by pilots, astronauts, and military personnel [10] suggests the potential applicability of individual aligners in aerospace medicine and extreme situation medicine.

The identified differences highlight the need to integrate dental care into training programs. The absence of significant differences in $VO_{2\ peak}$ and peak power may indicate that a greater level of impact of dental diseases at submaximal exertion levels, which are characteristic of prolonged training and competitions in martial arts. This aligns with data from [9], which revealed up to a 21% decrease in performance among athletes with poor oral health.

CONCLUSION

The presence of a dental diagnosis is associated with increased heart rate at rest, at the anaerobic threshold, and during the recovery phase, as well as with changes in power output at the anaerobic threshold. These changes may be attributed to pain, systemic inflammation, and nutritional disturbances, highlighting the systemic nature of the problem. The regression model demonstrated high predictive power, identifying dental diseases as an independent factor influencing HR_{AerT}, alongside gender, weight, and other medical diagnoses.

In our study, a significant impact of dental diseases on physical performance and endurance was identified. The presence of a dental diagnosis (Group 1) was associated with statistically significant differences ($p < 0.05$) compared to the group of athletes without a dental diagnosis (Group 0) across a range of physiological indicators characterizing physical endurance and performance: respiratory exchange ratio $R(0) = 1.05$ [1.03; 1.09], $R(1) = 1.04$ [1.03; 1.07]; heart rate at aerobic threshold $HR_{AerT}(0) = 110.00$ [100.00; 122.00], $HR_{AerT}(1) = 114.00$ [102.00; 126.00]; heart rate at anaerobic threshold $HR_{AT}(0) = 143.00$ [132.00; 154.00], $HR_{AT}(1) = 147.00$ [134.00; 158.00]; heart rate at peak load $HR_{peak}(0) = 151.00$ [144.00; 160.00], $HR_{peak}(1) = 152.00$ [144.00; 163.00]; heart rate at 3 min of recovery $HR_{3min}(0) = 91.00$ [82.00; 101.00], $HR_{3min}(1) = 93.00$ [84.00; 102.00]; power output at the

anaerobic threshold level $Pwr_{AT}(0)$ = 190.00 [165.00; 230.00], $Pwr_{AT}(1)$ = 200.00 [165.00; 240.00].

The identified patterns indicate the need to incorporate dental examinations and preventive measures into mandatory medical support for athletes. Regular checkups, timely treatment of oral diseases, and the use of protective devices, such as mouthguards, can mitigate the negative impact of dental pathologies on athletic performance. Based on statistically significant differences in performance parameters among combat sports athletes, measures have been proposed to develop interventions for correcting the dental status of highly qualified athletes. These include:

1) need for prevention (regular dental examinations, the use of protective mouthguards, and increased awareness among athletes and coaches about oral hygiene are key measures to minimize negative impacts);

2) comprehensive approach (integrating dental care into the athletes' medical support system should become a mandatory part of preparation, particularly in contact sports);

3) further research (additional studies are needed to assess the long-term effects of dental interventions and their impact on cognitive functions and psychological state of athletes);

4) use of individual aligners (to prevent excessive impact on teeth under conditions of overload and extreme situations.

These findings and recommendations can serve as a basis for developing prevention programs and improving athletic performance through optimizing oral health. The use of individual aligners is recommended for specialists whose activities involve overload and extreme situations (pilots, astronauts, military personnel). Individual aligners are considered as a means of prevention and adaptation to real and simulated conditions of changing gravity, relevant to the development of adequate methods for preventing the negative impact of space flight factors.

References

1. Gallagher J, Ashley P, Petrie A, Needleman I. Oral health and performance impacts in elite and professional athletes. *Community Dentistry and Oral Epidemiology*. 2018;46(6):563–8. https://doi.org/10.1111/cdoe.12392
2. Teixeira KG, Bodanese A, Bandeira JKP, Rezende M. The importance of sports dentistry in the athlete's performance. *Research, Society and Development*. 2021;10(3):e51510313683. https://doi.org/10.33448/rsd-v10i3.13683
3. Needleman I, Ashley P, Fine P, Haddad F, Loosemore M, de Medici A, et al. Oral health and elite sport performance. *British Journal of Sports Medicine*. 2015;49(1):3–6. https://doi.org/10.1136/bjsports-2014-093804
4. Needleman I, Ashley P, Fine P, Haddad F, Loosemore M, de Medici A, et al. Consensus statement: Oral health and elite sport performance. *The British Dental Journal*. 2014;217(10):587–90. https://doi.org/10.1038/sj.bdj.2014.1000
5. Soileau KM, Le A, Bresky I. Effects of oral health problems in athletic performance. *International Journal of Preventive and Clinical Dental Research*. 2024;11(1):10–5. https://doi.org/10.4103/ijpcdr.ijpcdr_6_24
6. El Ouali EM, Zouhal H, Bahije L, Ibrahimi A, Benamar B, Kartobou J, et al. Effects of malocclusion on maximal aerobic capacity and athletic performance in young sub-elite athletes. *Sports*. 2023;11(3):71. https://doi.org/10.3390/sports11030071
7. Ashley P, Di Iorio A, Cole E, Tanday A, Needleman I. Oral health of elite athletes and association with performance: a systematic review. *British Journal of Sports Medicine*. 2015;49(1):14–9. https://doi.org/10.1136/bjsports-2014-093617
8. Tripodi D, Cosi A, Fulco D, D'Ercole S. The impact of sport training on oral health in athletes. *Dentistry Journal*. 2021;9(5):51. https://doi.org/10.3390/dj9050051
9. Needleman I, Ashley P, Petrie A, Fortune F, Turner W, Jones J, et al. Oral health and impact on performance of athletes participating in the London 2012 Olympic Games: a cross-sectional study. *British Journal of Sports Medicine*. 2013;47(16):1054–8. https://doi.org/10.1136/bjsports-2013-092891
10. Kahatab R, Polyzois I. Enhancing athletic performance: the critical role of oral health in sports. *Evidence-Based Dentistry*.2025;26:105–6. https://doi.org/10.1038/s41432-025-01157-0
11. Schulze A, Busse M. Sports Diet and oral health in athletes: a comprehensive review. *Medicina*. 2024;60(2):319. https://doi.org/10.3390/medicina60020319
12. Pappas E, Nightingale EJ, Simic M, Ford KR, Hewett TE, Myer GD. Do exercises used in injury prevention programmes modify cutting task biomechanics? A systematic review with meta-analysis. *British Journal of Sports Medicine*. 2015;49(10):673–80. https://doi.org/10.1136/bjsports-2014-093796
13. Munteanu SE, Scott LA, Bonanno DR, Landorf KB, Pizzari T, Cook JL, et al. Effectiveness of customised foot orthoses for Achilles tendinopathy: a randomised controlled trial. *British Journal of Sports Medicine*. 2015;49(15):989–94. https://doi.org/10.1136/bjsports-2014-093845
14. Crighton AJ. Oral medicine in children. *British Dental Journal*. 2017;223(9):706–12. https://doi.org/10.1038/sj.bdj.2017.892
15. Pan Y, Tam JM, Tsoi JK, Lam WY, Huang R, Chen Z, et al. Evaluation of laboratory scanner accuracy by a novel calibration block for complete-arch implant rehabilitation. *Journal of Dentistry*. 2020;102:103476. https://doi.org/10.1016/j.jdent.2020.103476
16. Okshah A, Vaddamanu SK, Khalid I, Kota MZ, Udeabor SE, Baig FAH. The impact of bruxism on athletic performance: a systematic review and meta-analysis. *International Journal of Sports Medicine*. 2025. https://doi.org/10.1055/a-2588-0766
17. Merle CL, Richter L, Challakh N, Haak R, Schmalz G, Needleman I, et al. Orofacial conditions and oral health behavior of young athletes: A comparison of amateur and competitive sports. *Scandinavian Journal of Medicine and Science in Sports*. 2022;32(5):903–12. https://doi.org/10.1111/sms.14143
18. Huttunen M, Kämppi A, Soudunsaari A, et al. The associa-

tion between dental caries and physical activity, physical fitness, and background factors among Finnish male conscripts. *Odontology*. 2023;111(1):192–200. https://doi.org/10.1007/s10266-022-00717-5
19. Sabharwal A, Stellrecht E, Scannapieco FA. Associations between dental caries and systemic diseases: a scoping review. *BMC Oral Health*. 2021;21(1):472. https://doi.org/10.1186/s12903-021-01803-w
20. Gomes TC, Gomes Moura JL, Baia-da-Silva DC, Lima RR, Rodrigues PA. Twenty-first century knowledge mapping on oral diseases and physical activity/exercise, trends, gaps, and future perspectives: a bibliometric review. *Frontiers in Sports and Active Living.* 2024;6:1410923. https://doi.org/10.3389/fspor.2024.1410923
21. Chan CCK, Chan AKY, Chu CH, Tsang YC. Physical activity as a modifiable risk factor for periodontal disease. *Frontiers in Oral Health*. 2023;4:1266462. https://doi.org/10.3389/froh.2023.1266462
22. Botelho J, Mascarenhas P, Viana J, Proença L, Orlandi M, Leira Y, et al. An umbrella review of the evidence linking oral health and systemic noncommunicable diseases. *Nature Communications*. 2022;13(1):7614. https://doi.org/10.1038/s41467-022-35337-8
23. Pavlin D, Anthony R, Raj V, Gakunga PT. Cyclic loading (vibration) accelerates tooth movement in orthodontic patients: A double-blind, randomized controlled trial. *Seminars in Orthodontics*. 2015;21(3):187–94. https://doi.org/10.1053/j.sodo.2015.06.005

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COMPARATIVE ASSESSMENT OF PROTEOMIC REGULATION OF BONE TISSUE DURING 21-DAY HEAD-DOWN BED REST (–6°) AND 21-DAY DRY IMMERSION

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Introduction. Experimental possibilities during actual spaceflight are limited, making ground-based models, such as dry immersion (DI) and head-down bed rest (HDBR) tests, highly relevant. Changes in bone tissue are induced by alterations in a complex set of environmental factors at the proteomic level, compensating for changes caused by reduced gravity and decreased motor activity. However, upon continued exposure, other regulatory circuits are activated.
Objective. Comparative assessment of proteomic regulation of bone tissue status in 21-day HDBR (tilted at 6°) and 21-day DI tests.
Materials and methods. Using mass spectrometry methods, plasma samples from 8 healthy male volunteer subjects (mean age 20–44 years) under the conditions of 21-day HDBR and 10 subjects (mean age 23–34 years) under 21-day DI were studied. The Perseus software was used for statistical analysis and identification of molecular functions and biological processes involving the proteins. The correspondence of major biological processes, according to gene ontologies specified in the GO database, and identified proteins was established using the knowledge base of the ANDSystem and STRING.
Results. Nine proteins with significantly altered levels on Day 21 of HDBR ($p < 0.05$) and eight proteins with significantly altered levels on Day 21 of DI ($p < 0.05$) were identified. These proteins are associated with biological processes occurring in bone tissue. Some of the identified proteins form stable protein–protein interaction (PPI) networks, indicating potential co-expression. Two common proteins — haptoglobin (Hp) and glutathione peroxidase (GPx) — were identified on Day 21 of both DI and HDBR.
Conclusions. The findings offer an insight into the proteomic mechanisms regulating biological processes in bone tissue of healthy individuals under the influence of 21-day HDBR and 21-day DI. Annotations for each protein involved in bone tissue biological processes during 21-day HDBR (tilted at 6°) and 21-day DI are provided. These results are of great importance for aerospace and clinical medicine.

Keywords: dry immersion; head-down bed rest; proteome; skeletal system; healthy volunteer subjects

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СРАВНИТЕЛЬНАЯ ОЦЕНКА ПРОТЕОМНОЙ РЕГУЛЯЦИИ СОСТОЯНИЯ КОСТНОЙ ТКАНИ В 21-СУТОЧНОЙ АНТИОРТОСТАТИЧЕСКОЙ ГИПОКИНЕЗИИ (-6°) И 21-СУТОЧНОЙ «СУХОЙ» ИММЕРСИИ

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Введение. Экспериментальные возможности во время реального космического полета ограничены, поэтому актуально использование наземных моделей, таких как «сухая» иммерсия (СИ) и антиортостатическая гипокинезия (АНОГ). Изменения костной ткани индуцируются изменением комплекса факторов внешней среды на протеомном уровне, компенсируя изменения, вызванные снижением гравитации и уменьшением двигательной активности, но в дальнейшем с продолжением воздействия включаются другие контуры регуляции.
Цель. Сравнительная оценка протеомной регуляции состояния костной ткани в 21-суточной антиортостатической гипокинезии (-6°) и 21-суточной «сухой» иммерсии.
Материалы и методы. Методами масс-спектрометрии исследовали образцы плазмы крови 8 здоровых испытателей-добровольцев мужчин (средний возраст 20–44 года) в условиях 21-суточной АНОГ и 10 испытателей (средний возраст 23–34 года) в условиях 21-суточной «сухой» иммерсии. Для статистического анализа и определения молекулярных функций и биологических процессов, в которых участвовали белки, применяли программный пакет Perseus. Соответствие основных биологических процессов, согласно генным онтологиям, указанным в базе данных GO, и определенных белков устанавливали с помощью базы знаний системы ANDSystem, STRING.

Результаты. Выявлено 9 белков с достоверно изменяющимся уровнем на 21-е сутки АНОГ ($p < 0,05$) и 8 белков с достоверно изменяющимся уровнем на 21-е сутки СИ ($p < 0,05$), связанных с биологическими процессами, протекающими в костной ткани. Часть выявленных белков связаны в устойчивые сети белок-белковых взаимодействий, то есть могут коэкспрессироваться. Выделены два общих белка (гаптоглобин и глутатионпероксидаза) на 21-е сутки СИ, 21-е сутки АНОГ.
Выводы. Полученные данные впервые обращают внимание на протеомные механизмы регуляции биологических процессов костной ткани у здоровых лиц под влиянием 21-суточной АНОГ и 21-суточной «сухой» иммерсии. Приведены аннотации каждого белка — участника биологических процессов в костной ткани в 21-суточной АНОГ (-6°) и 21-суточной «сухой» иммерсии. Эти результаты имеют большое значение для авиакосмической и клинической медицины.

Ключевые слова: «сухая» иммерсия; антиортостатическая гипокинезия; протеом; костная система; здоровые испытатели-добровольцы

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INTRODUCTION

The knowledge of biological changes in bone tissue induced by prolonged exposure to microgravity, as a component of the set of spaceflight (SF) factors, is of paramount importance for space agencies planning to conduct deep space exploration missions.

Weightlessness causes physiological changes affecting the musculoskeletal system and interrelated sensory, neuromuscular, vascular, and other processes. In view of the limited experimental opportunities during actual spaceflight, ground-based models [1] can be used to assess the effects of microgravity, to identify gravitational mechanisms regulating the body's physiological systems, and to elucidate adaptation mechanisms to weightlessness [1].

Comparisons of the results obtained by ground-based model experiments (head-down bed rest, HDBR; dry immersion, DI) and those during actual SF have demonstrated the utility of ground-based simulations of specific SF factors for studying fundamental patterns and changes in the organism. The use of these models has become essential for research purposes, allowing for a broader participant pool and enhanced scientific significance through the analysis of various protocols (e.g., different durations or use of countermeasures) [2]. Notably, the use of invasive procedures (e.g., venipuncture for blood sampling, biopsies) and the logistics of cargo delivery to the orbit and back to the Earth present significant challenges [2], further emphasizing the importance of ground-based model studies.

Extended knowledge in this field is crucial for the social and economic aspects of maintaining the health, work capacity, and social activity of astronauts. Research into the effects of physical inactivity and forced bed rest on human health is highly relevant in the context of modern health issues and population aging [2]. Reduced mechanical loading on the skeleton, caused by bed rest and/or spaceflight, leads to bone mass loss, reflected in a decrease in the bone mineral density (BMD) both throughout the entire skeleton and in specific sites, such as the spine, femoral neck, and tibia [3, 4].

Baran et al. and Man et al. identified structural changes in the radius and tibia using peripheral quantitative computed tomography (pQCT) and high-resolution peripheral quantitative computed tomography (HR-pQCT) under spaceflight conditions [5, 6].

Alterations in hormonal status accompanying bone resorption and formation processes have been noted in several studies. For instance, disrupted circadian rhythms of somatotropin and a decrease in its average daily concentration during a 370-day HDBR experiment were described [7]. Grigoriev et al. and Austermann et al. demonstrated changes in the levels of hormones regulating calcium metabolism during both 120-day and 370-day HDBR. Specifically, by day 75 of the experiment, parathyroid hormone (PTH) levels were below baseline, while the calcitonin (CT) concentration was elevated [8, 9]. Inoue et al. established that insulin-like growth factor I (IGF-I), its binding protein (IGFBP), and insulin-like growth factor binding protein 3 (IGFBP-3) increased during 120-day bed rest, suggesting potential IGF-I resistance in bones under reduced mechanical load and strain [10].

Although model studies cannot fully replicate the conditions experienced by astronauts during SF, there is a notable similarity in the pathophysiological changes observed during prolonged mobility restriction (bed rest) and the issues faced by astronauts [2].

In DI tests, the lack of mechanical support for specific body areas during immersion creates a state akin to weightlessness, termed unloading, which induces

physiological changes in the musculoskeletal and other bodily systems [1]. Hypokinesia and hypodynamia are primary characteristics of physical inactivity induced by DI. Hypodynamia implies reduced postural muscle load, while hypokinesia represents a decrease in motor activity. In addition to the acute restriction of normal muscle activity and reduced load on muscles and bones, thermoneutral immersion rapidly induces a significant decline in muscle tone and tension [11, 12], which is unattainable even with prolonged bed rest models.

Kotov et al. found that after seven days of DI, the bone mineral density (BMD) in the lower skeleton (proximal femoral epiphysis) decreased by 2%, while the density in the upper body (skull, hand, rib bones) was approximately 2% higher than the baseline values. Moreover, three weeks of recovery after DI were sufficient to reverse these bone density changes [13]. It is hypothesized that these changes are a secondary effect of the cranial fluid shift to the upper body, where increased hydrostatic pressure promotes the movement of ions and proteins into the bone. Thus, DI appeared to exert a similar effect as HDBR on bone resorption in specific body regions. Baecker et al. showed that markers of bone resorption increased as early as day 2 of bed rest [14]. Kotov et al. also confirmed the rapid onset of bone tissue degradation under immobilization conditions, such as DI or HDBR.

According to Markin et al., biochemical processes involved in bone formation were not affected by day 7 of DI, as evidenced by the lack of changes in serum alkaline phosphatase (ALP) concentration [13, 15], serum procollagen type I N-terminal propeptide (PINP), and bone-specific alkaline phosphatase (BAP). Markers of bone resorption, such as tartrate-resistant acid phosphatase (TRAP) and urinary C-terminal telopeptide of type I collagen (CTX), showed a slight increase during a 7-day DI [16]. However, Markin et al. did not detect any changes in the activity of total acid phosphatase as a biomarker of osteoclast activity during a 7-day DI [15].

It is evident that alterations in bone tissue are induced by a complex set of environmental factors at the proteomic level, initially compensating for reduced gravity and decreased motor activity. However, upon prolonged exposure, additional regulatory pathways become engaged.

In this research, we aim to carry out a comparative assessment of proteomic regulation of bone tissue status under the conditions of 21-day head-down bed rest (tilted at –6°) and 21-day dry immersion.

MATERIALS AND METHODS

The 21-day head-down bed rest (HDBR) study involved eight healthy male volunteers aged 20–44 years. The participants were maintained at a –6° head-down tilt position relative to the horizontal for 21 days under controlled conditions at the MEDES Research Center as part of the joint Russian-French CaDy WEC laboratory program (Toulouse, France, 2014). No countermeasures to prevent adaptive physiological changes were implemented. The participants received a standardized diet with controlled nutrient content and monitored water intake. Blood samples were collected prior to the study (baseline) and on day 21 of HDBR.

The 21-day dry immersion (DI) study involved 10 healthy male volunteers aged 23–34 years, approved by the IBMP RAS Medical Expert Commission. All participants provided their written informed consent (IBMP RAS Bioethics Committee Protocol No. 483, 03.08.2018). The study complied with the requirements of Helsinki Declaration for participant safety and risk management. The experiment was conducted at IBMP RAS using the Dry Immersion facility, part of the “Medical and Technical Complex for Innovative Space Biomedicine Technologies” research facility (RSF grant No. 19-15-00435). Proteomic research was supported by state assignment No. FMFR-2024-0032. Both studies used comparable diets and hydration protocols.

Plasma samples were collected at identical timepoints in both studies, including seven days prior to exposure (baseline) and on day 21 of DI/HDBR. Blood was drawn from the cubital vein (5 mL) into EDTA tubes after fasting. Samples were centrifuged in 9 mL K_3 EDTA vacuum tubes at 3000 rpm (MPW-350R centrifuge, Poland) for 10 min at 4°C. The preparation technique and the subsequent chromatography-mass spectrometry analysis were identical for all biological samples, regardless of the experimental exposure factor, in order to ensure the validity of the comparison of the results obtained in both experiments.

FASP filters were used for sample preparation. Mass spectrometry analysis was performed using a MaXis 4G spectrometer (Bruker Daltonics, Germany) equipped with the MaxQuant software. Peak lists included up to eight major peaks per 100 Da window. The SwissProt database (forward/reverse) with 10 ppm precursor mass tolerance was used for identification purposes. Peptides were identified with ≥7 amino acids, FDR (false discovery rate) 0.01, and the “match between runs” option.

Statistical analysis was conducted using the Perseus software with Mann–Whitney U tests for small samples [17]. Functional annotation was carried out using the ANDSystem and STRING databases with GO term enrichment[1].

RESULTS AND DISCUSSION

The results of a comparative proteomic analysis of statistically significant differentially expressed proteins in ground-based model studies is presented in the Table.

The analysis of the data presented in the Table reveals statistically significant differences ($p < 0.05$) in protein levels compared to the baseline values under

[1] ANDSystem Knowledge Base. https://www-bionet.sysbio.cytogen.ru/and/cell/#!/app/about

21-day HDBR conditions. These include increased levels of APOE, Hp, complement C5 alpha chain, GPx3, HCII, and decreased levels of CD146 antigen, AngII, and CHLE. Meanwhile, under 21-day DI conditions, statistically significant increases were observed in PHLD, PON1, TTHY, TRFE, VTNC, as well as Hp and GPx3 compared to the baseline values.

Angiotensinogen (*AGT* gene) is involved in bone remodeling regulation. The renin-angiotensin-aldosterone system (RAAS) is known to participate in bone tissue regulation. Angiotensin II activates osteoclasts through increased expression of receptor activator of nuclear factor κ-B ligand (RANKL) on osteoblasts, leading to reduced BMD. The use of angiotensin II receptor blockers is associated with lower rates of bone fractures [18]. Some evidence suggests that RAAS blockade may reduce the risk of osteoporotic fractures [19]. However, other studies indicate that RAAS blockers do not reduce and may even increase the incidence of osteoporotic fractures [20]. RAAS components are expressed in bone tissue, activating local RAAS responses that lead to decreased bone density [21]. Angiotensinogen increased interleukin-6 secretion in vitro and reduced alkaline phosphatase activity only in otosclerotic cells. These observations suggest a connection between local renin-angiotensin system activity and otosclerosis, suggesting new therapeutic possibilities.

Mineralocorticoid receptors have also been identified in human osteoblasts, osteoclasts, and bone cells. Previous research indicates that local bone RAAS plays an important role in various causes of osteoporosis. RAAS blockers may reduce BMD loss through cascades involving angiotensin II type 1 receptor (AT1R), the ligand-receptor system (OPG/RANKL), and angiotensin-converting enzyme 2 (ACE2/Ang)(1-7)/Mas. By restoring bone physicochemical properties and reducing fracture

Table. Comparative assessment of proteomic regulation of bone tissue status during 21-day head-down bed rest (–6°) and 21-day dry immersion

Protein names	Genes	21-day HDBR		21-day DI	
		Protein levels in % relative to baseline	Level of confidence in the observed changes (*p*-value)	Protein levels in % relative to baseline	Level of confidence in the observed changes (*p*-value)
Apolipoprotein E (APOE)	*APOE*	104.2	0.0024	–	–
Haptoglobin (Hp)	*HP*	105.3	0.0047	107.8	0.000018
Complement C5 alpha chain	*C5*	115.1	0.0014	–	–
CD146 antigen (MUC18)	*MCAM*	95.7	0.0020	–	–
Extracellular glutathione peroxidase (GPx3)	*GPX3*	106.5	0.0084	113.6	0.0000023
Angiotensinogen (AngII)	*AGT*	96.1	0.0035	–	–
Heparin cofactor II (HCII)	*SERPIND1*	108.1	0.0038	–	–
Cholinesterase (CHLE)	*BCHE*	94.3	0.0039	–	–
Phosphatidylinositol-glycan-specific phospholipase D (PHLD)	*GPLD1*	–	–	113.2	0.000015
Serum paraoxonase (PON1)	*PON1*	–	–	115.2	0.00005
Fibronectin (FINC)	*FN1*	–	–	108.7	0.00000013
Transthyretin (TTHY)	*TTR*	–	–	105.0	0.000013
Serotransferrin (TRFE)	*TF*	–	–	109.6	0.0000018
Vitronectin (VTNC)	*VTN*	–	–	109.9	0.0000065

Table prepared by the authors using their own data

Note: "–" the data are not presented due to the lack of statistically significant differences.

risk, RAAS blockers could serve as an effective adjuvant therapy for osteoporosis [21].

Heparin cofactor II HCII (*SERPIND1* gene) stimulates osteogenic activity. Studies on HCII effects have investigated bone formation models stimulated by human tumors. HCII induced new bone growth over the cranial surface, even at a distance from the tumor mass. This suggests bone growth induction through growth factor production and the combined action of multiple factors on bone tissue [22].

Cholinesterase (*BCHE* gene) is an esterase with a broad substrate specificity. Acetylcholinesterase inhibitors (AChEIs) are known to stimulate acetylcholine receptors and are used in the treatment of Alzheimer's disease, providing protection against osteoporosis and inhibiting osteoclast differentiation and function. AChEIs variably reduced RANKL-induced transcription of nuclear factor of activated T cells 1 (Nfatc1) and osteoclast marker gene expression (primarily donepezil and rivastigmine, rather than galantamine). Additionally, AChEIs differentially inhibited RANKL-induced MAPK signaling, accompanied by reduced acetylcholinesterase transcription. Finally, AChEIs protected against OVX-induced bone loss primarily by inhibiting osteoclast activity. Collectively, AChEIs (mainly donepezil and rivastigmine) positively affected bone protection by suppressing osteoclast function through MAPK and Nfatc1 signaling pathways via downregulation of acetylcholinesterase [23].

Apolipoprotein E (*APOE* gene) is a biomarker of fracture risk and an indicator of lower BMD in patients with osteoporosis. The *APOE2* and *APOE4* alleles have been associated with lower BMD, as well as with higher levels of serum C-terminal telopeptide and urinary deoxypyridinoline, which are biomarkers of bone resorption. Codominance of the *APOE3* allele was also associated with fewer bone fractures in these patients over a 5-year follow-up period [24]. Apolipoprotein E levels were shown to increase significantly on day 7 of a space flight [25].

During the study under HDBR conditions, 33 significantly altered proteins were identified. Nine of these are associated with biological processes occurring in bone tissue (Fig. 1) and showed statistically significant changes compared to the baseline values. The proteins AGT and AGT II are annotated together as they are local regulators of bone tissue, belonging to the same group of angiotensin system regulators. Consequently, they are bioinformatically grouped by the ANDVisio software.

The results of 21-day HDBR exposure revealed statistically significant associations between specific proteins and key bone tissue biological processes. The ossification process demonstrated connections with four proteins, i.e., MUC18, GPX3, AngII, and APOE. Osteoblast differentiation was associated with one protein (Complement C5 alpha chain), while bone mineralization correlated with AngII levels. Bone development involved two proteins — AngII and CHLE, and bone resorption was linked to two proteins — AngII and Hp. Osteoclast differentiation similarly involved two proteins (AngII, Hp), bone growth was associated with one protein (HCII), and bone remodeling regulation correlated with AngII.

Our study also identified protein–protein interaction networks related to bone tissue regulation under 21-day HDBR conditions, with the corresponding data presented in Fig. 2. Six proteins — apolipoprotein E, haptoglobin, complement C5 alpha chain, angiotensin, cholinesterase, and extracellular glutathione peroxidase — formed an interconnected protein–protein interaction network suggesting potential co-expression. One protein (CD146 antigen) remained outside this network (Fig. 2). The constructed network reflects the mutual influence of these proteins on common bone tissue targets.

The protein interactions indicate coordinated regulation of bone metabolic pathways during mechanical unloading, with angiotensin II (AngII) emerging as a central regulator across multiple processes, including ossification, mineralization, and bone resorption. The network analysis provides insights into potential molecular mechanisms underlying HDBR-induced bone adaptation and suggests targets for countermeasure development.

Bergdolt et al. demonstrated that the complement protein C5 receptor (C5aR1) plays a crucial role in bone metabolism and fracture healing, being highly expressed on immune and bone cells, including osteoblasts and osteoclasts. C5aR1 induces osteoblast migration, cytokine production, and osteoclastogenesis. C5aR1 signaling in osteoblasts may potentially influence the RANKL/OPG signaling pathway balance that regulates bone tissue homeostasis, involving the receptor activator of nuclear factor κ-B ligand (RANKL) and osteoprotegerin (OPG), leading to increased bone resorption. Binding to the C5AR1 receptor triggers various responses, including intracellular calcium release, smooth muscle contraction, increased vascular permeability, and histamine release from mast cells and basophilic leukocytes [26]. Ignatius et al. suggested that complement may enhance the inflammatory response of osteoblasts and increase osteoclast formation, particularly in pro-inflammatory environments such as during bone healing or inflammatory bone diseases [27].

Pimenta-Lopes et al. established that genetic deletion of C5ar1, the receptor for the anaphylatoxin C5a, or treatment with a C5AR1 inhibitor reduced monocyte chemotaxis and osteoclast differentiation, partially preventing bone loss and osteoclastogenesis during chemotherapy or ovariectomy. Thus, inhibition of alternative complement pathways may have specific therapeutic effects in osteopenic disorders [28]. Meanwhile, Kunimatsu et al. showed that the cell surface glycoprotein MUC18 (*MCAM* gene) acts as a surface receptor that triggers tyrosine phosphorylation of FYN and a temporary increase in intracellular calcium concentration. This protein stimulates a cell population capable of bone

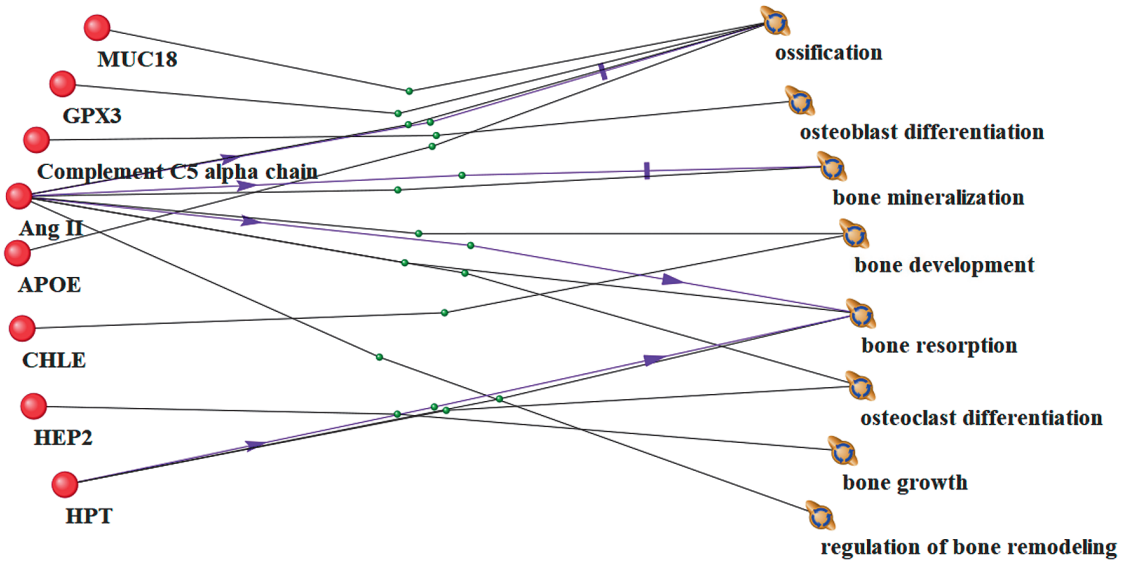

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Fig. 1. Interrelationship between significantly altered proteins and bone tissue processes under 21-day head-down bed rest (HDBR) conditions. Lines of different colors and labels indicate relationships between phenomena in peer-reviewed literature: black lines represent co-mention in scientific publications and association with biological processes; purple lines indicate stimulation or enhancement of biological process activity; green lines denote involvement or participation in biological processes, showing functional engagement in pathway mechanisms

formation and transendothelial migration *in vivo*, inducing bone tissue regeneration [29].

Thus, the identified changes indicate the involvement of both systemic and local protein regulators of bone tissue status in the biological processes of bone metabolism. Notably, the local bone RAAS plays an important role in the development of osteoporosis of various etiologies already in the early stages of exposure to the set of simulated SF factors. RAAS blockers may reduce BMD loss through AT1R, OPG/RANKL, ACE2/Ang (1-7)/Mas cascades.

In turn, AChEI inhibition exerts a positive effect on bone protection by suppressing osteoclast function through MAPK and Nfatc1 signaling pathways via downregulation of AChE. Changes in apolipoprotein E levels may reflect the activation of a protective biological process of osteogenesis in response to the duration of HDBR exposure.

An analysis of the blood plasma proteome on day 21 of DI identified 31 proteins with significantly altered levels, out of which eight proteins were associated with the regulation of biological processes in bone tissue, such as bone remodeling (serum paraoxonase), osteoclast differentiation (haptoglobin and transthyretin), bone mineralization (transthyretin), bone regeneration (transthyretin, fibronectin), osteoblast differentiation (fibronectin), resorption (transthyretin, serotransferrin), BMP-4 signaling pathway (fibronectin), and bone biosynthesis, including bone growth and development processes (fibronectin) (Fig. 3).

These proteins form a stable protein–protein interaction network (Fig. 4). The analysis of protein–protein interactions related to bone tissue regulation under 21-day DI conditions are presented in Fig. 4. Seven proteins, including haptoglobin, extracellular glutathione peroxidase, serum paraoxonase, serotransferrin, transthyretin, vitronectin, and fibronectin, form a

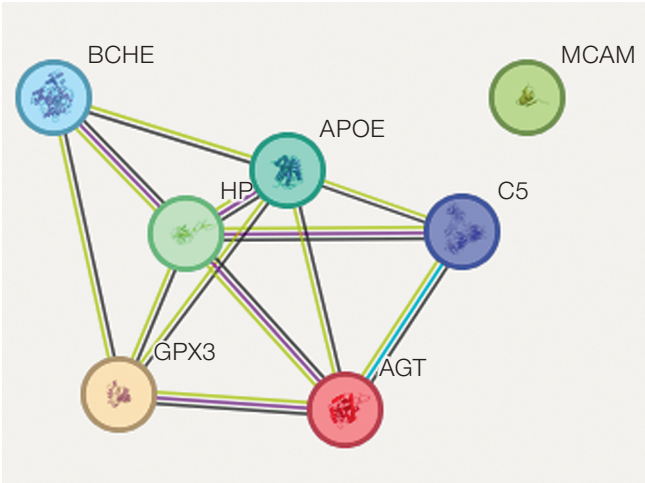

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Fig. 2. Protein–protein interactions associated with the regulation of bone tissue processes during 21-day head-down bed rest (HDBR). Protein–protein interaction lines are color-coded to indicate different types of evidence: light green represents co-mention in PubMed abstracts, indicating textual co-occurrence in scientific literature; crimson denotes experimentally determined interactions validated through laboratory methods; black indicates protein co-expression observed in transcriptomic or proteomic studies; light blue represents interactions curated from established biological databases

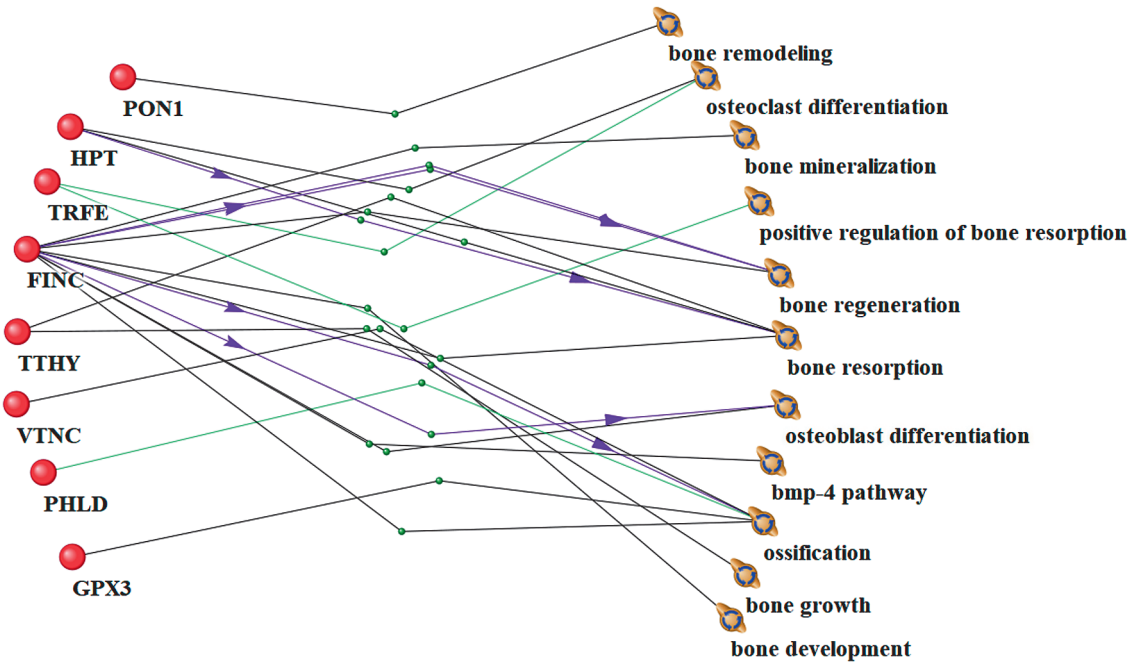

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Fig. 3. Interrelationship between significantly altered proteins and bone tissue processes under 21-day dry immersion (DI) conditions. Lines of different colors and labels indicate evidence-based relationships between biological phenomena: black lines represent co-occurrence in scientific literature and association with biological processes; purple lines indicate stimulation or enhancement of biological process activity; green lines denote functional involvement or participation in biological processes

protein–protein interaction network, indicating potential co-expression, while one protein (phosphatidylinositol-glycan-specific phosphatase) remains outside this network (Fig. 4). The constructed network reflects the mutual influence of these proteins on common bone tissue targets.

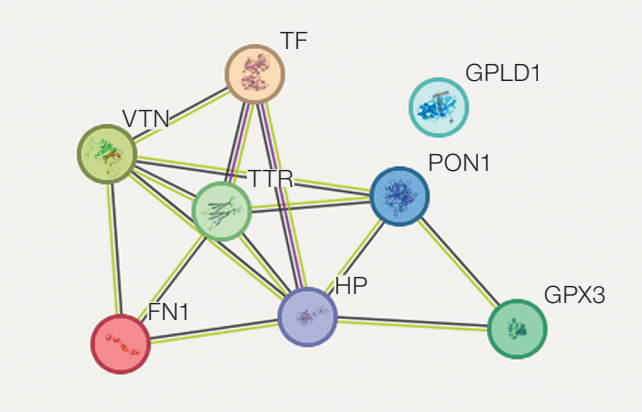

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Fig. 4. Protein–protein interactions associated with regulation of bone tissue processes during 21-day dry immersion (DI). Protein colors are assigned randomly by the visualization software. Interaction lines are color-coded as follows: light green indicates co-mention in PubMed abstracts (text mining evidence); crimson represents experimentally validated interactions; black denotes protein co-expression patterns

Fibronectin (*FN1* gene) participates in osteoblast condensation through matrix assembly via fibronectin fibrillogenesis, regulates type I collagen deposition by osteoblasts, and acts as a ligand for the immunoglobulin-like receptor family membrane protein (LILRB4), inhibiting monocyte activation. Fibronectin fibrillogenesis is involved in bone mineralization processes. Specific regulation of *FN1* during different phases of osteoblast differentiation has been established.

In studies by Xiong et al., fibronectin-1, thrombospondin-1, and biglycan were identified as key bone mineralization genes, with their upregulation associated with potential disturbances in bone remodeling processes. Fibronectin-1 (*FN1*), thrombospondin-1 (*THBS1*), and biglycan (*BGN*) were determined as the most significant genes in treating non-union fractures, highlighting the crucial role of *FN1*, *THBS1*, and *BGN* in extracellular matrix mineralization dynamics and bone regeneration [30]. Increased *FN1* expression promotes fracture healing by activating the TGF-β/PI3K/Akt signaling pathway [31].

Serotransferrin (*TF* gene) is involved in regulating biological processes of bone resorption and osteoclast differentiation. Higher levels of soluble transferrin receptor (sTfR) correlate with a lower trabecular number, cortical thickness, and cortical pore diameter. The relationship between the tibial bone density and strength and low

circulating concentrations of bone resorption and formation markers with serotransferrin levels likely results from the direct role of iron ions in collagen synthesis [32, 33]. Serotransferrin levels in dry blood spot extracts from cosmonauts were significantly altered after three and six months of SF [25]. This indicates that ground-based model studies do reproduce some proteomic biological processes of bone tissue regulation observed at different time points during SF [11].

Vitronectin (*VTN* gene) is present throughout the mineralized bone matrix of cancellous and cortical bones, suggesting its participation in bone remodeling through bone formation, resorption, and osteogenesis biological processes. Vitronectin is known to interact with glycosaminoglycans and proteoglycans, inhibiting the membrane-damaging effect of the terminal cytolytic complement pathway. Vitronectin deficiency was shown to increase osteoclast numbers and reduce total femoral bone volume in an ovariectomized mouse osteoporosis model [34]. Our previous studies noted that vitronectin levels significantly decreased after six months of SF [25], confirming the role of vitronectin in regulating osteogenesis and bone remodeling under DI and SF conditions in overall bone volume formation.

Serum paraoxonase/arylesterase 1 (*PON1* gene) plays an important role in maintaining the buffering colloidal properties of intervertebral discs. Low levels of *PON1* expression were established as a predictor of nucleus pulposus degeneration in intervertebral discs. Inflammation and oxidative stress can deteriorate the cellular environment of the nucleus pulposus, leading to intervertebral disc degeneration. Paraoxonase is an enzyme with anti-inflammatory and antioxidant effects. Aydın et al. investigated *PON1* expression in 88 human intervertebral disc samples and rat models, measuring tumor necrosis factor (TNF-α), interleukin (IL-1β), mitosuperoxide (SOX), aggrecan, and collagen II levels in nucleus pulposus cells. *PON1* expression was significantly suppressed in degenerative human and rat intervertebral discs. *PON1* levels were significantly reduced in degenerative cell models induced by TNF-α and oxidative stress (H_2O_2). TNF-α and interleukin-1β (IL-1β) levels clearly increased, while aggrecan and collagen expression decreased in cells transfected with *PON1* siRNA. *PON1* levels were also significantly higher in patients with osteoporotic hip fractures, particularly intertrochanteric femoral fractures and femoral neck fractures, compared to the control group [35].

Thus, low *PON1* expression is a predictor of severe intervertebral disc dysfunction. *PON1* plays a crucial role in maintaining the homeostatic balance of the intervertebral disc nucleus pulposus. Therapeutic approaches targeting *PON1* may be beneficial for alleviating nucleus pulposus dysfunction in the future.

Phosphatidylinositol-glycan-specific phospholipase D (*GPLD1* gene) primarily functions to hydrolyze the inositol phosphate bond in phosphatidylinositol glycan-anchored proteins, releasing these proteins from the membrane. Additionally, associations between the phosphatidylinositol-glycan-specific phospholipase locus and alkaline phosphatase levels were established, suggesting the specificity of this protein for bone tissue [36].

Transthyretin (*TTR* gene), TTHY is a transport protein involved in regulating biological processes of bone resorption and growth. Transthyretin levels gradually decrease with a reduction in BMD in osteoporosis patients [37]. Similar changes in the levels of this protein were identified in the dry blood spots of cosmonauts after six months of SF [25].

Two common proteins were identified regarding their participation in the biological processes of bone tissue regulation, observed both following 21 days of HDBR and 21 days of DI. These proteins are haptoglobin (Hp) and glutathione peroxidase (GPx3) (Fig. 5).

When examining the participation of these proteins in regulating the biological processes of bone tissue, the following observations should be mentioned. Thus, altered haptoglobin concentrations (*Hp* gene) were detected at day 21 in both DI and HDBR exposure conditions. Haptoglobin participates in regulating osteoclast differentiation and bone resorption. The protein Zscan10 is likely to be involved in regulating haptoglobin transcription during osteoclast differentiation. *In vitro* studies by Yanagihara et al. into the effects of human haptoglobin on bone resorption and prostanoid formation demonstrated that haptoglobin transcription negatively regulates osteoclast differentiation and modifies bone resorption processes [38]. Importantly, a proteomic analysis of the dry blood spots of cosmonauts after three months of SF also revealed significant changes in haptoglobin levels [25].

Our study identified altered concentrations of glutathione peroxidase 3 (*GPx3* gene) at day 21 under both DI and HDBR conditions. This protein protects structural elements of bone tissue cells from oxidative damage by catalyzing the reduction of hydrogen peroxide, lipid peroxides, and organic hydroperoxides with glutathione, thereby shifting the balance between osteoblast and osteoclast activity. According to Föger-Samwald et al., increased expression of SOD2 and GPX3 suggests enhanced antioxidant activity in bone samples from individuals with osteoporosis and hip fractures [39]. It is hypothesized that osteoclast production of reactive oxygen species suppresses protective mechanisms of natural antioxidants. Concomitant oxidative stress may lead to bone loss and, consequently, to osteoporosis development [40].

Notably, when comparing proteomic results from ground-based experiments (using identical materials, blood plasma, and sampling timepoints) and prolonged SF (dried blood spot extracts, different sampling timepoints), common proteins were identified across different timepoints (7 days, 3 and 6 months) of a long-duration SF [18], and in blood plasma samples analyzed at day 21 of HDBR and DI. These

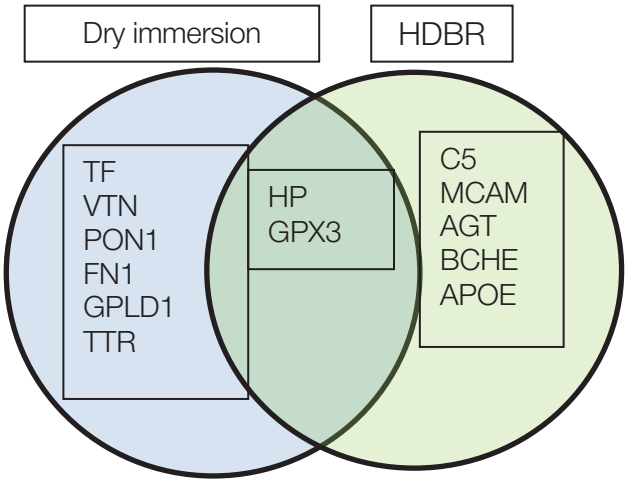

Figure prepared by the authors using their own data

Fig. 5. Common and differentially expressed proteins involved in bone tissue regulation under 21-day head-down bed rest (HDBR) and 21-day dry immersion (DI) conditions

include haptoglobin, apolipoprotein E, transthyretin, serotransferrin, and vitronectin. Their participation in bone tissue biological processes has been described above.

CONCLUSION

In the present study, we conducted a comparative assessment of proteomic regulation in bone tissue during 21-day head-down bed rest (tilted at 6°) and 21-day dry immersion tests. Proteomic investigations of bone tissue regulation mechanisms in ground-based model studies identified nine proteins with significantly altered levels under HDBR conditions and eight proteins with statistically significant changes under DI conditions, all associated with the regulation of biological processes in bone tissue (osteogenesis, osteoblast differentiation, osteoclast differentiation, resorption, bone mineralization, bone development, and bone remodeling regulation).

Several proteins (in both HDBR and DI studies) formed protein–protein interaction networks, indicating potential co-expression. Different protein networks were associated with distinct biological effects of antiorthostatic hypokinesia and DI of the same duration. Two common proteins were identified — haptoglobin (Hp) and glutathione peroxidase 3 (GPx3) — participating in the regulation of bone tissue biological processes on day 21 of both DI and HDBR. *Hp* transcription negatively regulates osteoclast differentiation and alters bone resorption processes. GPx3 protects structural elements of bone tissue cells from oxidative damage, shifting the balance between osteoblast and osteoclast activity.

Bone system metabolism is a complex process involving numerous mechanisms. The proteomic level of regulation examined in our study extends the current understanding of the mechanisms underlying bone tissue changes at specific timepoints (day 21) of HDBR and DI. This duration is not sufficiently long for osteopenia to develop in healthy subjects; however, proteomic regulation of bone tissue biological processes significantly changes during this period. These findings indicate the involvement of both systemic and local protein regulators in bone tissue metabolic processes. For the first time, we revealed that the local bone renin-angiotensin-aldosterone system (RAAS) plays an important role in regulating bone tissue biological processes by day 21 of model studies. RAAS blockers may reduce the loss of bone mineral density (BMD) through AT1R, OPG/RANKL, and ACE2/Ang (1–7)/Mas cascades. Inhibition of acetylcholinesterase (AChEI) positively influences bone protection by suppressing osteoclast function through MAPK and Nfatc1 signaling pathways via downregulation of AChE. Changes in apolipoprotein E levels may reflect the activation of a protective biological process of osteogenesis in response to the duration of HDBR exposure.

Notably, significant alterations were observed in transport proteins involved in regulating biological processes of both bone resorption and growth. The production of reactive oxygen species (ROS) by osteoclasts and the role of oxidative stress in bone mass loss were also highlighted. Changes in the proteomic regulation of mineralization in the cancellous and cortical bone matrix are of great importance. The state of the matrix determines bone remodeling through structure formation, resorption, and osteogenesis.

Our findings draw attention to the primary proteomic mechanisms regulating biological processes in bone tissue in healthy individuals under exposure to 21-day HDBR and 21-day DI. These results hold significant implications for aerospace and clinical medicine.

References

1. Navasiolava NM, Custaud MA, Tomilovskaya ES, Larina IM, Mano T, Gauquelin-Koch G, et al. Long-term dry immersion: review and prospects. *European Journal of Applied Physiology.* 2011;111(7):1235–60. https://doi.org/10.1007/s00421-010-1750-x
2. Hajj-Boutros G, Sonjak V, Faust A, Balram S, Lagace JC, St-Martin P, et al. Myths and Methodologies: Understanding the health impact of head down bedrest for the benefit of older adults and astronauts. Study protocol of the Canadian Bedrest Study. *Experimental Physiology.* 2024;109(5):812–87. https://doi.org/10.1113/EP091473
3. Hargens AR, Vico L. Long-duration bed rest as an analog to microgravity. *Journal of Applied Physiology.* 2016;120(8):891–903. https://doi.org/10.1152/japplphysiol.00935.2015

4. Oganov VS, Rakhmanov AS, Novikov VE, Zatsepin ST, Rodionova SS, Cann Ch. The state of human bone tissue during space flight. *Acta Astronautica.* 1991;23:129–33. https://doi.org/10.1016/0094-5765(91)90109-i
5. Baran R, Wehland M, Schulz H, Heer M, Infanger M, Grimm D. Microgravity-related changes in bone density and treatment options: a systematic review. *Internation Journal of Molecular Sciences.* 2022;23(15):8650. https://doi.org/10.3390/ijms23158650
6. Man J, Graham T, Squires-Donelly G, Laslett AL. The effects of microgravity on bone structure and function. *NPJ Microgravity.* 2022;8(1):9. https://doi.org/10.1038/s41526-022-00194-8
7. Larina IM, Morukov BV, Grigoriev AI. Circadian rhythms of human mineralotropic hormones during prolonged antiorthostatic hypokinesia. *Human Physiology.* 1999;25(6):89–95 (In Russ.).
8. Grigoriev AI, Morukov VV. 370-day antiorthostatic hypokinesia (tasks and general structure of the study). *Space Biology and Aerospace Medicine.* 1989;23(5):47–50 (In Russ.).
9. Austermann K, Baecker N, Zwart SR, Fimmers R, Stehle P, Smith SM, et al. Effects of antioxidant supplementation on bone mineral density, bone mineral content and bone structure in healthy men during 60 days of 6° head-down tilt bed rest: Results from a randomised controlled trial. *Nutrition Bulletin.* 2023;48(2):256–66. https://doi.org/10.1111/nbu.12619
10. Inoue M, Tanaka H, Moriwake T, Oka M, Sekiguchi C, Seino Y. Altered biochemical markers of bone turnover in humans during 120 days of bed rest. *Bone.* 2000;26(3):281–6. https://doi.org/10.1016/s8756-3282(99)00282-3
11. Tomilovskaya E, Shigueva T, Sayenko D, Rukavishnikov I, Kozlovskaya I. Dry immersion as a ground-based model of microgravity physiological effects. *Frontiers in Physiology.* 2019;10:284. https://doi.org/10.3389/fphys.2019.00284
12. Saveko A, Bekreneva M, Ponomarev I, Zelenskaya I, Riabova A, Shigueva T, et al. Impact of different ground-based microgravity models on human sensorimotor system. *Frontiers in Physiology.* 2023;14:1085545. https://doi.org/10.3389/fphys.2023.1085545
13. Kotov SA, Oganov VS, Skripnikova IA. *Imitation of the early effects of weightlessness in human bone tissue in conditions of head down bed rest and dry immersion.* Proceedings of the conference of young scientists. Moscow; 2003 (In Russ.).
14. Baecker N, Tomic A, Mika C, Gotzmann A, Platen P, Gerzer R, et al. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. *Journal of Applied Physiology.* 2003;95(3):977–82. https://doi.org/10.1152/japplphysiol.00264.2003
15. Markin AA, Morukov BV, Zhuravleva OA, Zabolotskaya IV, Vostrikova LV, Lyapunova NA, et al. Dynamics of blood biochemical parameters in an experiment with 7-day immersion. *Aerospace and Environmental Medicine.* 2008;42:56–9 (In Russ). EDN: RBLSWZ
16. Linossier MT, Amirova LE, Thomas M, Normand M, Bareille MP, Gauquelin-Koch G, et al. Effects of short-term dry immersion on bone remodeling markers, insulin and adipokines. *PLoS One.* 2017;12(8):e0182970. https://doi.org/10.1371/journal.pone.0182970
17. Brzhozovskiy AG, Kononikhin AS, Pastushkova LC, Kashirina DN, Indeykina MI, Popov IA, et al. The effects of spaceflight factors on the human plasma proteome, including both real space missions and ground-based experiments. *International Journal of Molecular Sciences.* 2019;20(13):3194. https://doi.org/10.3390/ijms20133194
18. Shukuri T, Nakai K, Tanaka S, Yamada S, Tokumoto M, Tsuruya K, et al. Angiotensin II receptor blockers and bone fracture in chronic kidney disease patients: the Fukuoka kidney disease Registry Study. *Clinical and Experimental Nephrology.* 2023;27(11):919–27. https://doi.org/10.1007/s10157-023-02385-3
19. Zhang Y, Wang L, Song Y, Zhao X, Wong MS, Zhang W. Renin inhibitor aliskiren exerts beneficial effect on trabecular bone by regulating skeletal renin-angiotensin system and kallikrein-kinin system in ovariectomized mice. *Osteoporosis International.* 2016;27:1083–92. https://doi.org/10.1007/s00198-015-3348-y
20. Zhang Y, Li XL, Sha NN, Shu B, Zhao YJ, Wang XL, et al. Differential response of bone and kidney to ACEI in db/db mice: A potential effect of captopril on accelerating bone loss. *Bone.* 2017;97:222–32. https://doi.org/10.1016/j.bone.2017.01.029
21. Mo C, Ke J, Zhao D, Zhang B. Role of the renin-angiotensin-aldosterone system in bone metabolism. *Journal of Bone and Mineral Metabolism.* 2020;38(6):772–9. https://doi.org/10.1007/s00774-020-01132-y
22. Izbicka E, Dunstan CR, Horn D, Harris S, Adams R, Mundy GR. Effects of human tumor cell lines on local new bone formation in vivo. *Calcified Tissue International.* 1997;60(2):210–5. https://doi.org/10.1007/s002239900216
23. Li S, Teguh D, Wu D, Hu C, Inderjeeth CA, Xu J. Antidementia medication acetylcholinesterase inhibitors have therapeutic benefits on osteoporotic bone by attenuating osteoclastogenesis and bone resorption. *Journal of Cellular Physiology.* 2023;238(8):1823–35. https://doi.org/10.1002/jcp.31057
24. Souza LS, Rochette NF, Pedrosa DF, Lopes Magnago RF, Freire Filho TB, Vieira FLH, et al. Role of APOE gene in bone mineral density and incidence of bone fractures in brazilian postmenopausal women. *Journal of Clinical Densitometry.* 2018;21(2):227–35. https://doi.org/10.1016/j.jocd.2017.03.005
25. Pastushkova LH, Goncharova AG, Kashirina DN, Polyakov DN, Larina IM. Identification of proteomic markers included in molecular networks of disorders in the skeletal system during long-term space flights. *Technologies of Living Systems*. 2025;22(1):5–21 (In Russ.).
26. Bergdolt S, Kovtun A, Hägele Y, Liedert A, Schinke T, Amling M, et al. Osteoblast-specific overexpression of complement receptor C5aR1 impairs fracture healing. *PLoS One.* 2017;12(6):e0179512. https://doi.org/10.1371/journal.pone.0179512
27. Ignatius A, Schoengraf P, Kreja L, Liedert A, Recknagel S, Kandert S, et al. Complement C3a and C5a modulate osteoclast formation and inflammatory response of osteoblasts in synergism with IL-1β. *Journal of Cellular Biochemistry.* 2011;112(9):2594–605. https://doi.org/10.1002/jcb.23186
28. Pimenta-Lopes C, Sánchez-de-Diego C, Deber A, Egea-Cortes A, Valer JA, Alcala A, et al. Inhibition of C5AR1 impairs osteoclast mobilization and prevents bone loss. *Molecular Therapy.* 2023;31(8):2507–23. https://doi.org/10.1016/j.ymthe.2023.04.022
29. Kunimatsu R, Rikitake K, Yoshimi Y, Putrani NAR, Hayashi Y, Tanimoto K. Bone differentiation ability of CD146-positive stem cells from human exfoliated deciduous teeth. *International Journal of Molecular Sciences.* 2023;24(4):4048. https://doi.org/10.3390/ijms24044048
30. Xiong W, Shu XL, Huang L, He SQ, Liu LH, Li S, et al. Bioinformatics analysis and experimental validation of differential genes and pathways in bone nonunions. *Biochemical Genetics.* 2024;62(6):4494–517. https://doi.org/10.1007/s10528-023-10633-0

31. Zhang H, Chen X, Xue P, Ma X, Li J, Zhang J. FN1 promotes chondrocyte differentiation and collagen production via TGF-β/PI3K/Akt pathway in mice with femoral fracture. *Gene.* 2021;769:145253. https://doi.org/10.1016/j.gene.2020.145253
32. Dakkumadugula A, Pankaj L, Alqahtani AS, Ullah R, Ercisli S, Murugan R. Space nutrition and the biochemical changes caused in Astronauts Health due to space flight: A review. *Food Chemistry: X.* 2023;20:100875. https://doi.org/10.1016/j.fochx.2023.100875
33. O'Leary TJ, Jackson S, Izard RM, Walsh NP, Carswell AT, Oliver SJ, et al. Iron status is associated with tibial structure and vitamin D metabolites in healthy young men. *Bone.* 2024;186:117145. https://doi.org/10.1016/j.bone.2024.117145
34. Nakashima M, Suzuki A, Hashimoto K, Yamashita M, Fujiwara Y, Miyamoto Y. Vitronectin regulates osteoclastogenesis and bone remodeling in a mouse model of osteoporosis. *Anatomy and Cell Biology.* 2024;57(2):305–15. https://doi.org/10.5115/acb.23.251
35. Aydın M, Avcı E. The role of oxidative stress and antioxidants in older individuals with osteoporotic hip fractures. *Turkish Journal of Trauma and Emergency Surgery.* 2025;31(1):9–14. https://doi.org/10.14744/tjtes.2024.89335
36. Cao J, Zhou A, Zhou Z, Liu H, Jia S. The role of GPLD1 in chronic diseases. *Journal of Cellular Physiology.* 2023;238(7):1407–15. https://doi.org/10.1002/jcp.31041
37. Li XS, Zhang JR, Zhao YL, Li Y, Sun Y, Liu T, et al. Reduced prealbumin is associated with bone mineral density in women with osteoporosis. *Nutrition.* 2017;33:338–42. https://doi.org/10.1016/j.nut.2016.08.002
38. Yanagihara Y, Inoue K, Saeki N, Sawada Y, Yoshida S, Lee J, et al. Zscan10 suppresses osteoclast differentiation by regulating expression of Haptoglobin. *Bone.* 2019;22:93–100. https://doi.org/10.1016/j.bone.2019.02.011
39. Föger-Samwald U, Vekszler G, Hörz-Schuch E, Salem S, Wipperich M, Ritschl P, et al. Molecular mechanisms of osteoporotic hip fractures in elderly women. *Experimental Gerontology.* 2016;73:49–58. https://doi.org/10.1016/j.exger.2015.11.012
40. Zhang W, Dong R, Diao S, Du J, Fan Z, Wang F. Differential long noncoding RNA/mRNA expression profiling and functional network analysis during osteogenic differentiation of human bone marrow mesenchymal stem cells. *Stem Cell Research and Therapy.* 2017;8(1):30. https://doi.org/10.1186/s13287-017-0485-6

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CURRENT ISSUES IN ASSESSING THE EXPECTED PROFESSIONAL LONGEVITY OF EMPLOYEES

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Introduction. The task of maintaining the professional longevity of employees, particularly in technology-intensive and potentially hazardous industries, is becoming increasingly relevant in the context of aging populations and increasing life expectancy. Existing methods for assessing occupational health are in many cases fragmented and fail to account for the entire set of physical, psychological, and social factors. In this article, we address this issue by developing an integral group index of professional longevity (IGIPL).
Objective. The development and implementation of the IGIPL as a tool for quantitative assessment of the level of professional longevity among nuclear industry employees, taking into account morbidity, health status, results of medical examinations and psychophysiological testing, stress levels, and engagement.
Materials and methods. A retrospective study covering the period of 2023–2024 was conducted. The analysis was based on depersonalized data from employees of VNIITF (Snezhinsk) and the Kalinin NPP (Udomlya). The study included HR reports on morbidity with temporary disability (TD), final reports of periodic medical examinations (PME), annual reports of psychophysiological examinations (PPE), as well as the results of corporate surveys on stress levels (SL) and emotional burnout (EB). We present only relative summary data, without considering working conditions in the index calculation. Standardized methods were used to assess the parameters, including the Perceived Stress Scale-10, the Burnout Assessment Tool, and the E.L. Notkin method for analyzing TD.
Results. The calculation of the IGIPL showed a positive trend. Thus, the index increased by 2.6 points at the Kalinin NPP (a rise from 69.6 to 72.2 points) and decreased at VNIITF (from 67.2 to 65.8 points). The key factor that had the most pronounced negative impact was the high rate of morbidity with temporary disability (1914 days per 100 workers at VNIITF). At the Kalinin NPP, an improvement in the distribution of employees by health groups and a decrease in the proportion of individuals with a high level of emotional burnout were recorded, indicating the effectiveness of the preventive measures implemented by the organization.
Conclusions. The IGIPL has proven its effectiveness as a tool for monitoring professional longevity and identifying risk areas. The study results underscore the necessity for comprehensive programs aimed at reducing morbidity, managing stress, and increasing employee engagement. The IGIPL methodology can be adapted for other industries. Its further elaboration will enhance the accuracy of assessments. The data obtained hold practical significance for developing corporate programs aimed at preserving health and extending the professional longevity of employees.

Keywords: occupational health; professional longevity; morbidity; medical examination; psychophysiological examination; stress; engagement

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АКТУАЛЬНЫЕ ВОПРОСЫ ОЦЕНКИ ОЖИДАЕМОГО ПРОФЕССИОНАЛЬНОГО ДОЛГОЛЕТИЯ РАБОТНИКОВ

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Введение. В современных условиях старения населения и увеличения продолжительности жизни актуальной становится проблема сохранения профессионального долголетия работников, особенно в высокотехнологичных и потенциально опасных отраслях. Существующие методы оценки профессионального здоровья часто фрагментарны и не учитывают комплексное влияние физических, психологических и социальных факторов. Для решения этой проблемы предлагается разработка интегрального группового индекса профессионального долголетия (ИГИПД).
Цель. Разработка и внедрение ИГИПД как инструмента количественной оценки уровня профессионального долголетия коллектива работников атомной отрасли с учетом заболеваемости, состояния здоровья, результатов медосмотров и психофизиологического обследования, уровня стресса и вовлеченности.
Материалы и методы. Проведено ретроспективное исследование с периодом охвата 2023–2024 гг. Объектом анализа были деперсонифицированные данные работников ВНИИТФ (г. Снежинск) и Калининской АЭС (г. Удомля). В исследование включены кадровые отчеты о заболеваемости с временной утратой трудоспособности (ВУТ), заключительные акты периодических медицинских

осмотров (ПМО), годовые отчеты психофизиологических обследований (ПФО), а также результаты корпоративных анкетирований уровня стресса (УС) и эмоционального выгорания (УЭВ). В статье приведены только относительные сводные данные; условия труда в расчет индекса не включались. Для оценки параметров применялись стандартизированные методики: «Шкала воспринимаемого стресса-10», «Burnout Assessment Tool», а также методика Е.Л. Ноткина для анализа ВУТ.

Результаты. Расчет ИГИПД показал положительную динамику: индекс претерпел повышение на 2,6 балла на Калининской АЭС (рост с 69,6 до 72,2 балла) и снижение показателя во ВНИИТФ (с 67,2 до 65,8 балла). Ключевым фактором, оказавшим наиболее выраженное негативное влияние, явился высокий уровень заболеваемости с временной утратой трудоспособности (1914 дней на 100 работников во ВНИИТФ). На Калининской АЭС зафиксировано улучшение распределения работников по группам здоровья и снижение доли лиц с высоким уровнем эмоционального выгорания, что указывает на эффективность реализуемых организацией профилактических мероприятий.

Выводы. ИГИПД доказал свою эффективность как инструмент для мониторинга профессионального долголетия и выявления зон риска. Результаты исследования подчеркивают необходимость комплексных программ, направленных на снижение заболеваемости, управление стрессом и повышение вовлеченности работников. Методология ИГИПД может быть адаптирована для других отраслей, а ее дальнейшее совершенствование позволит повысить точность оценок. Полученные данные имеют практическую значимость для разработки корпоративных программ по сохранению здоровья и продлению профессионального долголетия работников.

Ключевые слова: профессиональное здоровье; профессиональное долголетие; заболеваемость; медицинский осмотр; психофизиологическое обследование; стресс; вовлеченность

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Финансирование: работа выполнена без спонсорской поддержки.

Соответствие принципам этики: исследование не требовало заключения биоэтического комитета, поскольку использовали деперсонифицированные данные работников двух предприятий атомной отрасли.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

In the current demographic context of an aging society driven by increasing life expectancy in Russia, as well as the need to address humanitarian and economic challenges, the task of preserving the professional longevity of employees is acquiring particular relevance. The growth in the retirement age and rising demands on qualifications and working conditions require new approaches to assessing and managing the professional activity of employees throughout their careers. The issue of extending professional longevity is inextricably linked to ensuring timely and accessible medical care, including prevention, diagnosis, and rehabilitation. These tasks are set by the national project “Long and Active Life”, the implementation of which began on 01.01.2025.

According to the WHO definition, “health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.[1]

At the beginning of the 21st century, due to the growing life expectancy of the population in economically developed countries, the WHO launched a program for active longevity (in the original — active ageing) [1], focusing mainly on the quality of life and maintaining the health of older people.[2] However, in the 2015 edition, the program shifted attention to the employment of older people. This shift is explained by growing concerns among governmental and business structures that population aging would place an unsustainable burden on the budgets of enterprises and social support institutions, primarily pension funds. In order to counter these threats, a series of urgent measures [2] were proposed to improve the economic situation, including measures to retain specialists in the labor market beyond the retirement age.[3,4]

Thus, concern for the working individual, beyond its humanitarian significance, has acquired a pronounced macroeconomic meaning. As a result, the concepts of “occupational health” and “professional longevity” have become the focus of close attention for specialists in medicine, occupational psychology, and related disciplines. Occupational health is defined as an integral characteristic of the “functional state of the human body based on physical and mental parameters, aiming to assess its ability for specific professional activity with given efficiency and duration over a specified life period, as

[1] Preamble to the Charter (Constitution) of the World Health Organization. https://apps.who.int/gb/bd/PDF/bd48/basic-documents-48th-edition-ru.pdf?ua=1#page=9 (request date of 07.04.2025).

[2] Active ageing: a policy framework; 2002. WHO reference number: WHO/NMH/NPH/02.8. https://extranet.who.int/agefriendlyworld/wp-content/uploads/2014/06/WHO-Active-Ageing-Framework.pdf (request date of 02.05.2025).

[3] World Health Organization (WHO). Project 1: Global Strategy and Action Plan on Ageing and Health. 2015. https://www.who.int/ageing/ageing-global-strategy-draft1-ru.pdf (request date of 15.08.2020).

[4] Multisectoral action for healthy ageing based on a life-cycle approach: draft global strategy and action plan on ageing and health. Report of the Secretariat of the Sixty-ninth session of the WHO World Health Assembly, 22 April 2016. https://apps.who.int/iris/bitstream/handle/10665/253277/A69_17-ru.pdf?sequence=1&isAllowed=y (request date of 05.02.2022).

well as its resistance to adverse factors accompanying this activity."[5]

The aforementioned WHO definition of health, which includes mental and social well-being, should be extended by the concept of occupational health, i.e., a person's ability to fully realize themselves as a qualified specialist, experience a sense of self-worth and engagement in the life of the team and the work process, acceptance and support from colleagues, as well as to feel cared by management.

Professional longevity is the "ability of a person to solve professional tasks at a high level throughout the entire period of labor activity allotted by society,"[6] "that is, to maintain occupational capacity [4, 8, 9]." Given the demographic situation, the importance of preserving this ability and the desire to continue working after reaching retirement age should be emphasized. Human health is influenced by a large number of social and economic factors, with the living and working conditions of citizens being of particular importance. Surveys of the working population have revealed that "more than a third lead a sedentary lifestyle, 56% regularly face overtime work, 54% of Russians report periodic stress at work, 10% report constant stress, and 45% say they have experienced professional burnout."[7]

The Russian government has developed a number of regulatory legal acts and national projects aiming to improve the health and increase the life expectancy of the country's population, as well as to overcome difficulties related to the demographic situation. Decree of the President of the Russian Federation No. 309[8] specifies national development goals for the period up to 2030 and with a perspective to 2036, among which the foremost are preserving the population, strengthening health, improving people's well-being, and supporting the family.

The current stage involves the implementation of the national projects for the next six years. Among them, the National Project[9] is aimed at increasing the population's life expectancy to 78 years by 2030. The plans include modernization of the primary healthcare system in Russia, development of measures for the prevention and early diagnosis of cancer, creation of a national digital platform "Health," and development of a medical rehabilitation system.

In order to ensure early and timely detection of chronic non-communicable diseases, which are the main cause of mortality (cardiovascular, oncological, and respiratory diseases, diabetes, etc.), preventive medical examinations and population health checkups are being carried out [4, 5].

Decree of the President of the Russian Federation No. 145[10] has declared a transition to personalized, predictive, and preventive medicine, high-tech healthcare, and health preservation technologies, including for the working population. Directive No. 830-r[11] outlines strategies for extending active healthy longevity, creating conditions for realizing the personal potential of elderly citizens, and expanding their participation in the society. Federal Law No. 311-FZ[12] has introduced comprehensive amendments to the Labor Code of the Russian Federation aimed at transforming approaches in the field of labor protection, implementing and developing a system for the prevention of occupational injuries and diseases, and improving mechanisms to incentivize employers to enhance working conditions.

Labor protection has become one of the priority areas of the Russian state policy, with the goal of creating a prosperous and safe environment for the citizens. There is a raising awareness among employers about the economic feasibility of promoting a healthy lifestyle among employees and encouraging regular preventive medical examinations and health checkups, as well as the importance of implementing special comprehensive programs to create appropriate conditions for employees to maintain a healthy lifestyle [7].

Directive of the Government of the Russian Federation No. 833-r[13] has approved a set of measures to incentivize employers and employees to improve working conditions and preserve the health of workers, as well as to motivate citizens to adopt a healthy lifestyle. These measures provide for the creation and replication of best corporate and regional practices for encouraging employers to enhance working conditions and preserve the health of workers.

While acknowledging the importance of the measures implemented by the Russian state to improve public policy in healthcare and labor protection for preserving the health of the working population, attention should be drawn to the persistently high mortality rate from non-communicable diseases, the prevalence of smoking and

[5] Health Psychology: Textbook for Universities / Edited by GS Nikiforov. — St. Petersburg: Peter, 2006.
[6] L.V. Mardakhaev Social Pedagogy: Textbook. Moscow: Gardariki, 2005.
[7] Federation Council of the Federal Assembly of the Russian Federation. Transcript of parliamentary hearings on the topic "Protecting the Health of the Working Population" dated 24.10.2024. http://council.gov.ru/activity/activities/parliamentary/161497/ (request date of 05.05.2025).
[8] Decree of the President of the Russian Federation No. 309 "On the National Development Goals of the Russian Federation for the Period until 2036" dated 07.05.2024. http://government.ru/docs/all/155078/
[9] National project "Long and Active Life". https://национальныепроекты.рф/new-projects/prodolzhitelnaya-i-aktivnaya-zhizn/
[10] Decree of the President of the Russian Federation No. 145 "On the Strategy for Scientific and Technological Development of the Russian Federation" dated 28.02.2024. http://www.kremlin.ru/acts/bank/50358
[11] Decree of the Government of the Russian Federation No. 830-r dated 07.04.2025, on the approval of the "Strategy for Action in the Interests of Older Citizens in Russia until 2030." http://government.ru/docs/54753/
[12] Federal Law No. 311-FZ "On Amendments to the Labor Code of the Russian Federation" dated 02.07.2021. http://www.kremlin.ru/acts/bank/46959
[13] Decree of the Government of the Russian Federation No. 833-r dated 26.04.2019, on the approval of the "Set of Measures to Stimulate Employers and Employees to Improve Working Conditions and Preserve the Health of Workers, as well as to Motivate Citizens to Lead a Healthy Lifestyle.". http://static.government.ru/media/files/ElHhjehSWOoZSfE4OuOTqJuF5mr7e7P7.pdf

alcohol consumption among the population, poor nutrition, insufficient physical activity, a formalistic attitude and low level of trust in health checkups. Psychosomatic diseases are becoming widespread, to a large extent being influenced by psychological factors, insufficient stress resistance, and prolonged psychoemotional tension.

In the context of demographic challenges and increasing demands to preserve labor potential, objective tools capable of assessing the health status of employees and their level of professional longevity are becoming particularly important. In order to address this task, we set out to develop a methodology for calculating an Integral Group Index of Professional Longevity (IGIPL) based on a set of medical, psychophysiological, and social parameters. The index enables the planning and monitoring of corporate and state programs aimed at extending professional activity, allows for tracking the dynamics of key parameters, and facilitates comparative analysis across different enterprises.

In this study, we aim to develop an IGIPL for use as a tool for quantitative assessment of the level of professional longevity among nuclear industry employees, taking into account morbidity, health status, results of medical examinations and psychophysiological testing, stress levels, and engagement.

To that end, we set the following objectives:

- to justify the IGIPL calculation methodology, including the selection of parameters and determination of their weight significance based on expert assessments;
- to calculate an IGIPL and carry out a comparative analysis of its values using the example of two nuclear industry enterprises for 2023 and 2024, with an assessment of parameter dynamics;
- to determine general approaches to developing corrective measures based on the IGIPL, aimed at increasing the level of professional longevity of employees.

MATERIALS AND METHODS

We carried out a retrospective and observational study covering the period of 2023–2024. The analysis was based on anonymized data from employees of two nuclear industry enterprises: VNIITF (Snezhinsk) and the Kalinin NPP (Udomlya).

The following information sources were used:

- Human Resources (HR) reports on morbidity with temporary disability (TD);
- final reports on the results of periodic medical examinations (PME) indicating the distribution of employees by health groups;
- annual reports of psychophysiological examinations (PPE);
- materials from corporate surveys assessing stress levels (SL) and emotional burnout (EB).

In this article, we present exclusively relative values based on aggregated, anonymized data. It should be noted that working conditions were not considered in the calculation of the IGIPL; therefore, their detailed analysis was beyond the scope of this study.

Standardized methodologies were used to assess specific parameters:

- SL was assessed using the “Perceived Stress Scale-10.” The maximum score on this scale is 50 points. To normalize the data (i.e., to convert the scale to a 100-point dimension), the obtained values were multiplied by two;
- EB level[14] was assessed using the “Burnout Assessment Tool (BAT),” specifically its version for working individuals. A short Russian-language version provided by the developer of the methodology was used [3];
- The E.L. Notkin method[15] was used to assess morbidity with TD, calculated as the number of days per 100 employees.

Thus, the combination of the above sources and methods enabled a comprehensive analysis of professional health parameters and the calculation of the IGIPL.

Weight coefficients (W) were established based on an expert assessment method aimed at determining the relative significance of various parameters for the professional longevity of employees. The study involved 20 experts in the fields of occupational medicine, psychophysiology, and human resource management. Data collection was carried out using standardized questionnaires followed by their statistical processing for the determination of valid weight coefficients (Table 1).

The assigned coefficients reflect the contribution of each parameter to the overall value of IGIPL. Experts attributed the greatest significance (coefficients of 0.20–0.15) to the following parameters: temporary disability (TD), health group (HG), stress level (SL), emotional burnout level (EB), and work engagement (E). According to specialists, these parameters most significantly determine the current state and dynamics of professional longevity, being directly linked to the risks of lost work capacity, decreased motivation, and premature departure from the profession.

Lower weight coefficients (0.10 each) were assigned to the parameters of fitness for work based on medical examinations (PME) and psychophysiological resilience (PPR). Despite their undeniable importance, experts considered these factors to play more of a supportive rather than a determining role in shaping professional

[14] Burnout Assessment Tool: version for working individuals. https://burnoutassessmenttool.be/wp-content/uploads/2020/11/BAT_Russian.pdf (request date of 03.05.2025).

[15] Methodological Guidelines MR 2.2.9.0375-25 “Analysis of the causes of temporary disability to identify priority professional groups for the development of medical and preventive measures”.

Table 1. Weight coefficients determined by experts

No.	Parameter	Abbreviation	Weighting coefficients (W)
1.	Temporary Disability	TD	0.20
2.	Health Group	HG	0.15
3.	Periodic Medical Examination	PME	0.10
4.	Psychophysiological Examination	PPE	0.10
5.	Stress Level	SL	0.15
6.	Emotional Burnout Level	EB	0.15
7.	Engagement	E	0.15

Table compiled by the authors based on their own data

Table 2. Temporary disability assessment scale (S_TD)

Temporary disability in days per 100 workers according to E.L. Notkin	Points on the S_TD assessment scale*
< 500	100
500–599	85
600–799	70
800–999	55
1000–1199	40
1200–1499	25
> 1500	10

Table compiled by the authors based on data from source MR 2.2.9.0375-25[16]

Note: * — the S_TD scale step is designed such that for every increase of 200–300 days of TD per 100 employees, the index value sequentially decreases by 15 points.

longevity, especially in the presence of other critical deviations.

The integration of the TD parameter was carried out through the S_TD assessment scale (Table 2). This approach normalizes the parameter to a 0–100 scale, reflects the generally accepted expert assessment according to the E.L. Notkin scale, and eliminates mathematical distortions at extreme values.

Therefore, the formula for calculating the Integral Group Index of Professional Longevity (IGIPL) takes the following form:

$$IGIPL = W\_TD \times S\_TD + W\_HG \times HG + \\ + W\_PME \times PME + W\_PPE \times PPE + + W\_E \times E + \\ + W\_SL \times (100 - SL) + W\_EB \times (100 - EB), \quad (1)$$

wherein:
W — the weight coefficient of the parameter;
S_TD — the score for morbidity with temporary disability;
HG — the proportion of employees in health groups I–II (%);
PME — the proportion of employees deemed fit for work without restrictions based on the results of periodic medical examinations (%);
PPE — the proportion of employees deemed fit based on the results of psychophysiological examinations (%);
E — the level of work engagement (%);
SL — the stress level according to the scale (points);
EB — the level of emotional burnout (%).

The IGIPL calculation was conducted using the example of enterprises of the State Corporation "Rosatom," which operate in technology-intensive and potentially hazardous industries: nuclear power and the defense-industrial complex.

The objects under analysis were the All-Russian Research Institute of Technical Physics (VNIITF, Snezhinsk) and the Kalinin Nuclear Power Plant

[16] Methodological Guidelines MR 2.2.9.0375-25 "Analysis of the Causes of Temporary Disability to Identify Priority Professional Groups for the Development of Medical and Preventive Measures".

Table 3. Interpretation of IGIPL values

Scores	Level	Interpretation of IGIPL Values
80–100	High	High level of professional longevity. Supportive measures for health protection, strengthening psychological well-being, and employee engagement are sufficient
60–79	Medium	Medium level of professional longevity. The implementation of regular preventive programs for stress management and maintaining employee motivation is required
40–59	Low	Low level of professional longevity. Targeted health-improvement and organizational measures aimed at improving the physical and psycho-emotional state of workers are necessary
< 40	Critical	Critical level of professional longevity. The implementation of comprehensive programs for health protection, workload reduction, and fostering a favorable work environment is required

Table compiled by the authors based on their own data

(Udomlya). These enterprises are characterized by a high degree of responsibility, complex technological processes, and stringent requirements for the health and psychophysiological resilience of their employees.

RESULTS AND DISCUSSION

The IGIPL is formed based on a set of various parameters. When growing, these parameters either contribute to the growth of professional longevity or, conversely, lead to its decrease. The key parameters influencing the IGIPL are presented below.

Factors whose growth has a positive effect on the IGIPL:

- Health Group (HG): The more employees belong to Health Groups I and II, the higher the overall level of resilience and physical readiness of the team to perform professional tasks.
- Fitness for Work based on Medical Examinations (PME): A high proportion of workers without medical restrictions reflects good overall health of the personnel and contributes to stable labor activity.
- Psychophysiological Resilience (PPE): The ability to maintain performance under difficult conditions is a crucial component for sustaining professional longevity.
- Work Engagement (E): A high level of engagement promotes professional stability, reduces the likelihood of burnout, and increases motivation.

Factors whose growth has a negative effect on the IGIPL:

- Temporary Disability (TD, days): An increase in the number of disability days indicates a decline in the overall health level of the team and negatively impacts the index value.
- Stress Level (SL): An elevated stress level leads to decreased performance, reduced psychological resilience, and a decline in the quality of professional duties.
- Emotional Burnout (EB): A high level of burnout is one of the primary factors for premature termination of employment.

The IGIPL was developed as a practical tool for employers to assess the level of professional longevity among employees at the enterprise level. The index can be used to formulate, implement, and subsequently evaluate the effectiveness of programs aimed at protecting employee health, increasing motivation, and improving the psychological climate within the team.

We performed IGIPL calculations based on the parameters of physical, psychological, and social well-being for the period of 2023–2024 in accordance with the developed methodology (Table 4).

According to the results obtained, the IGIPL values at VNIITF decreased over the year by 1.4 points (from 67.2 to 65.8 points). However, at the Kalinin NPP, the index increased by 2.6 points (from 69.6 as of 2023 to 72.2 points in 2024). Both organizations were characterized by an average level of IGIPL (60–79 points), which reflects a satisfactory but insufficiently stable state of the professional longevity level of employees. A slight decrease in the parameter was noted at VNIITF, while a positive trend was observed at the Kalinin NPP, likely associated with the effectiveness of the implemented measures to strengthen occupational health and improve working conditions.

Our analysis identified key parameters that determine the value of IGIPL. The most significant factor was morbidity with temporary disability (TD). The VNIITF enterprise exhibited a critically high level of morbidity with TD: 1914 days per 100 workers in 2023 and 1912 days in 2024. According to the E.L. Notkin scale, this corresponds to the minimum score (10 points) and indicates an area of chronic overload and unfavorable health status of personnel, requiring immediate corrective measures.

Table 4. Calculation of IGIPL for each enterprise for 2023–2024

Organization	Year	S_TD	W_TD × S_TD	W_HG × _HG	W_PME × PME	W_PPE × PPE	W_SL × (100 – SL)	W_EB × (100 – EB)	W_E × E	IGIPL
VNIITF	2023	10	2.0	3.3	9.92	10.0	13.9	13.9	14.1	67.2
VNIITF	2024	10	2.0	4.05	9.88	10.0	13.2	13.2	13.5	65.8
Kalinin NPP	2023	25	5.0	4.5	8.84	10.0	13.6	13.3	14.2	69.6
Kalinin NPP	2024	25	5.0	6.88	9.18	10.0	13.6	13.9	13.5	72.2

Table compiled by the authors based on their own data

Note: W — the weight coefficient of the parameter; TD — temporary disability; HG — health groups; PME — periodic medical examination; PPE — psychophysiological examinations; SL — stress levels; EB — emotional burnout.

At the Kalinin NPP, this parameter was somewhat lower (1321 and 1222 days per 100 workers in 2023 and 2024, respectively), which corresponds to 25 points and also indicates a significant loss of working capacity.

The second most significant factor is the distribution of employees by health groups. At the VNIITF enterprise, a moderate increase in the proportion of employees with Health Groups I–II was observed over time (from 22% in 2023 to 27% in 2024); however, the baseline level remains low. At the Kalinin NPP, a pronounced positive shift was observed: from 30% in 2023 to 45.9% in 2024, reflecting a more effective implementation of preventive and health-improvement measures.

The results of PME showed high values at both enterprises. For instance, at VNIITF, over 98% of employees were cleared for work without restrictions, indicating strict control of professional suitability. At the Kalinin NPP, this parameter was somewhat lower (88–91%), although remaining within acceptable standards.

Psychophysiological examination (PPE) at both enterprises showed a 100% clearance of employees for further work. This result indicates the low informativeness of the parameter "proportion of employees suspended based on PPE results." However, the signs of strain and impaired psychophysiological adaptation identified during the examination are significant predictors of somatic ill-health and require consideration when planning preventive measures. In the future, it is advisable to use an integral assessment of psychophysiological adaptation, e.g., by indicating the proportion of employees with pronounced impairments.

The conducted analysis of psychoemotional factors revealed differences between the organizations. The stress level among workers at VNIITF was elevated, ranging 7–12 points, which should be considered an alarming signal and a prerequisite for developing targeted preventive measures. At the Kalinin NPP, this parameter remained stable (around 9 points), corresponding to a moderate level requiring regular monitoring.

The dynamics of emotional burnout also differed. At VNIITF, its prevalence increased from 7% to 12%, which, combined with rising stress levels, indicated a growing risk of deterioration in the psychoemotional state of workers. Conversely, at the Kalinin NPP, a decrease in the proportion of workers with a high level of burnout was recorded, from 11% to 7%, reflecting positive trends and the need to maintain them further.

The engagement index remained high at both enterprises (VNIITF: 94% → 90%; Kalinin NPP: 95% → 90%), which is a factor of resilience to stress and the likelihood of staff turnover. The slight decrease in engagement at VNIITF in the setting of increasing stress and burnout can be considered an early sign of professional exhaustion among the workforce.

As a result of the conducted research, a comprehensive assessment of professional longevity at two enterprises of the State Corporation "Rosatom" was obtained. We established that both organizations are within the stable range of IGIPL, indicating the stability of labor potential despite the presence of risk factors.

However, differences in dynamics were identified. Positive shifts were recorded at the Kalinin NPP, driven by a decrease in morbidity with temporary disability, a reduction in the prevalence of emotional burnout, and a significant increase in the proportion of employees with Health Groups I–II according to medical checkup data. Conversely, at the VNIITF enterprise, a critically high level of morbidity with temporary disability persisted, which is a key limiting factor for professional longevity. Furthermore, unfavorable changes in the psychoemotional state of the workforce were noted, including an increase in stress levels, a rise in the proportion of employees showing signs of burnout, and a decrease in engagement.

The obtained results underscore the necessity for long-term observation (at least 4–5 years) to confirm the identified trends and to form sustainable management strategies. At the same time, the IGIPL has proven its scientific and practical significance as an integral parameter. It demonstrates high sensitivity to key risks in the occupational environment and enables an objective assessment of the effectiveness of corporate and state programs aimed at preserving health and extending the professional longevity of personnel.

CONCLUSION

The Integral Group Index of Professional Longevity serves as a tool for monitoring the effectiveness of measures aimed at preserving the health of the working population and extending professional longevity. Its application will enhance the objectivity of assessing programs implemented by enterprises and facilitate the development of more targeted preventive measures against key risk factors.

The implementation of IGIPL-based targeted measures may contribute to extending the active working life of employees, reducing morbidity and professional burnout, and increasing labor productivity. Undoubtedly, a competent personnel policy must account for the negative impact of biological aging on psychophysiological functions.

The proposed algorithm for IGIPL calculation is a dynamic model that can be refined with new accumulated data on the influence of various factors on professional longevity.

Effective use of the IGIPL requires a comprehensive approach, including studying successful experience of other organizations, analyzing domestic and international scientific research in the field of occupational health, developing methodological recommendations tailored to industry specifics, assessing necessary resources, and creating a system for monitoring the effectiveness of implemented measures. The conducted research and the proposed algorithm can serve as a basis for forming a program aimed at enhancing the professional longevity of employees, reducing losses associated with temporary disability, and increasing motivation and job satisfaction. Companies that invest in employee health, adaptation, and psychological support see a 5–10% increase in efficiency and reduced costs associated with staff turnover. Thus, professional longevity is not merely the length of a career but a strategic factor for productivity growth in the context of labor shortage.

References

1. Bukhtiyarov IV. Current state and main directions of preserving and promoting health of the working population of Russia. *Russian Journal of Occupational Health and Industrial Ecology*. 2019;59(9):527–32 (In Russ.). https://doi.org/10.31089/1026-9428-2019-59-9-527-532
2. Rudnicka E, Napierała P, Podfigurna A, Męczekalski B, Smolarczyk R, Grymowicz M. The World Health Organization (WHO) approach to healthy ageing. *Maturitas*. 2020;139:6–11. https://doi.org/10.1016/j.maturitas.2020.05.018
3. Gantman AA, Gorblyansky YuYu, Kontorovich EP, Ponamareva OP. The concept of healthy aging at work. *Medical Herald of the South of Russia*. 2022;13(4):5–13 (In Russ.). https://doi.org/10.21886/2219-8075-2022-13-4-5-13
4. Kalache A, Gatti A. Active ageing: a policy framework. *Advances in Gerontology*. 2003;11:7–18. EDN: PKIVFP
5. Bloom DE, Chatterji S, Kowal P, Lloyd-Sherlock P, McKee M, Rechel B, et al. Macroeconomic implications of population ageing and selected policy responses. *Lancet*. 2015;385(9968):649–57. https://doi.org/10.1016/S0140-6736(14)61464-1
6. Allenov AM, Vasilyeva TP, Starostin IV, Makarova EV, Vorobeva AV. Factors that determine professional longevity in researchers. *Russian Journal of Occupational Health and Industrial Ecology*. 2021;61(6):385–401 (In Russ.). https://doi.org/10.31089/1026-9428-2021-61-6-385-401
7. Shestakova NN, Dzhanelidze MG, Skvortsova MB. Prospects for building a society for all ages in Russia: approaches to the study of the problem. *Bulletin of Education and Science Development of the Russian Academy of Natural Sciences*. 2021;25(2):57–70 (In Russ.). https://doi.org/10.26163/RAEN.2021.91.24.008
8. Shkarin VV, Vorobyev AA, Adzhienko VL, Andryushchenko FA. Professional longevity — ways and means of achievement. *Bulletin of Volgograd State Medical University*. 2022;19(2):19–26 (In Russ.). https://doi.org/10.19163/1994-9480-2022-19-2-19-19-26
9. Maslennikova GYa, Oganov RG. Prevention of noncommunicable diseases as an opportunity to increase life expectancy and healthy longevity. *Cardiovascular Therapy and Prevention*. 2019;2:5–12 (In Russ.). https://doi.org/10.15829/1728-8800-2019-2-5-12
10. Ustinova O.Yu., Zaitseva N.V., Vlasova E.M., Kostarev V.G. Corporate programs for preventing health disorders among workers employed at adverse productions as a tool for occupational risk management. *Health Risk Analysis*. 2020;2:72–82 (In Russ.). https://doi.org/10.21668/health.risk/2020.2.08.eng
11. Schaufeli WB, De Witte H, Desart S. Burnout Assessment Tool (BAT)—Development, Validity, and Reliability. *International Journal of Environmental Research and Public Health*. 2020;17:9495. https://doi.org/10.3390/ijerph17249495
12. Sorokin GA, Kir'yanova MN. Age dynamics of work capacity and fatigue risk in workers of industrial enterprises, education and healthcare. *Occupational Medicine and Industrial Ecology*. 2021;61(5):311–7 (In Russ.). https://doi.org/10.31089/1026-9428-2021-61-5-311-317

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DIAGNOSTIC SIGNIFICANCE OF SUBCLINICAL EPILEPTIFORM ACTIVITY IN PATIENTS WITH ALZHEIMER'S DISEASE

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Introduction. The high prevalence and significant disability of patients with Alzheimer's disease (AD) necessitate the search for new markers of disease progression and novel treatment approaches. Recent evidence is increasingly attracting the research attention to the value of electroencephalography (EEG) in detecting epileptiform activity in this patient population.
Objective. Detection of the frequency of epileptiform activity in patients with AD and evaluation of its clinical and diagnostic significance.
Discussion. EEG, in particular, prolonged sleep-deprived EEG, is capable of detecting subclinical epileptiform activity, which is associated with more severe cognitive impairments and contributes to disease progression. This review examines research data on the prevalence and clinical significance of subclinical epileptiform activity in AD patients without an epilepsy diagnosis. It also highlights key pathophysiological mechanisms linking epileptiform activity to the progression of cognitive decline in AD. Furthermore, it addresses the rationale for prescribing specific antiepileptic therapy upon detection of subclinical epileptiform activity.
Conclusions. The high clinical significance of performing electroencephalography and detecting epileptiform activity in patients with Alzheimer's disease, due to its potential negative impact on the progression of cognitive impairments and increased risks of developing epileptic seizures, has been demonstrated.

Keywords: neurodegenerative disease; Alzheimer's disease; electroencephalography; video-EEG monitoring; subclinical epileptiform activity; antiepileptic drugs; epilepsy

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ДИАГНОСТИЧЕСКАЯ ЗНАЧИМОСТЬ СУБКЛИНИЧЕСКОЙ ЭПИЛЕПТИФОРМНОЙ АКТИВНОСТИ У ПАЦИЕНТОВ С БОЛЕЗНЬЮ АЛЬЦГЕЙМЕРА

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Введение. Высокая распространенность и значительная инвалидизация пациентов с болезнью Альцгеймера (БА) требуют поиска новых маркеров прогрессирования этого заболевания, а также новых подходов к его лечению. В последнее время накапливается все больше сведений о значимости применения электроэнцефалографии у пациентов с БА с целью выявления эпилептиформной активности у данной категории.
Цель. Определение частоты встречаемости эпилептиформной активности у пациентов с БА и оценка ее клинической и диагностической значимости.
Обсуждение. Установлено, что проведение ЭЭГ, особенно продолженной, с включением сна, позволяет выявить субклиническую эпилептиформную активность, которая ассоциирована с более выраженными когнитивными нарушениями и способствует прогрессированию заболевания. В обзоре рассмотрены данные исследований по распространенности и клинической значимости субклинической эпилептиформной активности у пациентов с БА без диагноза «эпилепсия». Также освещены основные патофизиологические механизмы взаимосвязи эпилептиформной активности и прогрессирования когнитивных нарушений в рамках БА. Кроме того, рассматривается вопрос о целесообразности назначения специфической противоэпилептической терапии при выявлении субклинической эпилептиформной активности.
Выводы. Показана высокая клиническая значимость проведения электроэнцефалографии и выявления эпилептиформной активности у пациентов с болезнью Альцгеймера вследствие ее потенциального негативного влияния на прогрессирование когнитивных нарушений и повышения рисков развития эпилептических приступов.

Ключевые слова: нейродегенеративное заболевание; болезнь Альцгеймера; электроэнцефалография; видео-ЭЭГ-мониторинг; субклиническая эпилептиформная активность; противоэпилептические препараты; эпилепсия

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and one of the most prevalent diseases in the elderly, affecting 10–20 million people worldwide [1]. The disease is characterized by the formation of neurofibrillary tangles and amyloid plaques in the brain, manifesting as progressive cognitive impairment. Annual direct and indirect costs associated with this disease amount to $100 billion, making the search for new diagnostic and therapeutic methods critically important. It is predicted that delaying disease progression by five years could reduce healthcare costs related to AD by half [2–5].

Despite significant advances in laboratory and genetic diagnostics, as well as modern neuroimaging methods (magnetic resonance imaging (MRI) of the brain with morphometry, positron emission tomography), diagnosing dementia-related diseases remains challenging and, in many cases, inaccessible due to the cost of examinations. Currently, electroencephalography (EEG) is not part of the standard examination protocol for patients with dementia, including those with AD. However, numerous literature sources report that pathological brain activity (e.g., slowing of the background rhythm or epileptiform activity) may be recorded during EEG in AD patients, which could exacerbate the progression of cognitive impairments and increase the risk of epileptic seizures, further disadapting patients with AD [6, 7].

EEG with functional tests is a simple diagnostic method that allows assessment of the bioelectrical activity of the brain. Non-epileptiform pathological activity, such as theta or delta slowing (regional/diffuse) of bioelectrical brain activity, is a common finding in AD patients during routine EEG [6]. There is evidence suggesting that increased relative power of theta oscillations may be an early sign preceding dementia, thus being an important biomarker of disease progression [8, 9].

The detection of epileptiform activity in AD patients holds a great significance. However, routine EEG is often insufficient for capturing epileptiform activity, since approximately 85% of standard EEG recordings fail to detect epileptiform activity even in AD patients with epileptic seizures [10]. This highlights the need for more sensitive methods, such as prolonged video-EEG monitoring including sleep, magnetoencephalography (MEG), or invasive electrode placement through the *foramen ovale* to identify this pathological activity [10]. Some authors emphasize the higher prevalence of epileptiform activity in AD patients compared to the healthy population, as well as its significance in the progression of cognitive impairments in neurodegenerative disease. Thus, this pathological activity may represent a promising target for intervention in treating cognitive impairments in AD.

The aim of the study is to detect the frequency of epileptiform activity in patients with AD and verify its clinical significance.

MATERIALS AND METHODS

The search for scientific literature was conducted in electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (PubMed, Google Scholar) languages. The search queries included the following keywords or their combinations: Alzheimer's disease, electroencephalography, video-EEG monitoring, subclinical epileptiform activity.

The inclusion criteria for publications were literature systematic reviews and meta-analyses with data on the prevalence of subclinical epileptiform activity in AD, the pathophysiology of its occurrence, and the relationship between the neurodegenerative process, epileptiform activity, and cognitive impairments. The exclusion criteria were publications covering theoretical models, abstracts, and conference materials. In total, 52 articles published from 1998 to 2024 were reviewed.

RESULTS AND DISCUSSION

Subclinical epileptiform activity (SEA) in patients with Alzheimer's disease (AD)

Subclinical epileptiform activity (SEA) typically refers to epileptiform activity detected in EEG in patients without a history of epileptic seizures. According to scientific research, data on the prevalence and diagnostic significance of epileptiform activity in patients with AD are limited and contradictory. The reviewed publications show a significant variability (ranging from 2% to 54%) in the reported prevalence of SEA among patients diagnosed with AD, likely due to substantial methodological differences across the studies (Table) [11].

The presence of epileptiform activity may exacerbate the progression of cognitive impairments in AD patients. Moreover, its detection may serve as a marker for the potential development of epileptic seizures. For example, Kang and Mendez et al. observed the

development of epilepsy in approximately 10–22% of AD patients [7, 12].

When investigating the prevalence and significance of epileptiform discharges in patients with various types of dementia, Liedorp et al. [15] found that routine 30-min EEG detected epileptiform activity (predominantly regional in the temporal areas) only in 2% of patients with AD, mild cognitive impairment (MCI) and in 1% of patients with other types of dementia. These rates are similar to those in the general population. Only in 10% of patients with dementia, whose EEG showed epileptiform activity, developed seizures later in the course of the disease [15].

The low detection rate of epileptiform activity in patients with AD prompted researchers to use additional *foramen ovale* EEG electrodes for its identification. Thus, Lam et al. applied *foramen ovale* electrodes and showed that subclinical epileptiform activity, predominant during sleep (affecting memory consolidation), can be detected in the early stages of AD, in the absence of changes in the routine scalp EEG [21]. This highlights the need for larger EEG studies using additional techniques, including *foramen ovale* recording, to determine the diagnostic value of EEG in clinical practice.

According to a number of studies, a significant prevalence of SEA has been detected in AD patients, which is likely associated with the use of prolonged video-EEG monitoring including during sleep. Recently, increasing attention has been paid to the presence of SEA in AD patients due to evidence of a more pronounced cognitive decline and a faster disease progression in patients with SEA compared to those without it [16, 18, 22, 23].

Thus, Horvath et al. analyzed SEA in 52 AD patients and detected significantly more frequent subclinical epileptiform discharges (54%) among this group compared to healthy elderly people of corresponding age (25%) [18]. SEA was detected predominantly in the temporal regions, mostly on the left side, with bitemporal and right-temporal epileptiform activity being less common. The vast majority of SEA episodes occurred during sleep, most frequently recorded during stages 2 and 3 of sleep, while fewer spikes were detected in stage 1 sleep. Moreover, the presence of SEA was associated with more severe cognitive impairments. Horvath et al. showed that in patients with AD combined with SEA, cognitive decline over the observation period (3 years) occurred 1.5 times faster than in patients without epileptiform activity [18]. According to Vossel et al., epileptiform activity was detected in 42.4% of patients with AD and only in 10.5% of individuals in the control group of corresponding age without cognitive impairments [16]. Patients with SEA showed a faster decline in executive functions and global cognition, as measured by the instrument of Mini-Mental State Examination (MMSE), averaging 3.9 points/year compared to 1.6 points/year in patients without SEA [16].

Nous et al. studied patients with different stages of AD (preclinical, MCI, dementia) using such various methods as prolonged EEG, 50-min MEG, and high-density EEG [11]. The prevalence of SEA in these patients was 31% compared to the control group (8%) without cognitive dysfunction. The frequency was increasing along with the disease progressed, i.e., in 50% of cases with developed dementia, in 27% with MCI, and in 25% at the preclinical stage of AD. Although the use of MEG did not lead to a more frequent detection of SEA in AD compared to prolonged EEG and high-density EEG, MEG significantly outperformed other methods in terms of spike detection rate per 50 min (epileptiform activity representation index). Furthermore, in AD patients, the presence of SEA was associated with more pronounced impairments in visuospatial functions and attention, as well as with a relatively larger volume of the left frontal, left temporal, and entorhinal cortex compared to patients without epileptiform activity [7].

Pathophysiological mechanisms of the relationship between epileptiform activity, neurodegenerative process, and cognitive impairment in Alzheimer's disease

A number of authors consider epileptiform activity to be part of the pathophysiological mechanisms leading to cognitive impairment in AD. The proposed mechanisms include compromised glutamatergic system, excitotoxicity-induced neurodegeneration, accelerated amyloid and tau protein deposition under the influence of epileptiform discharges, remodeling due to increased excitability leading to functional network disconnection, and sleep architecture changes [23].

One hypothesis describes a vicious cycle where molecular changes in AD promote neuronal hyperexcitability [24], which in turn exacerbates the neurodegenerative process in AD [25]. It was reported that in AD, soluble oligomeric Aβ (amyloid-beta), rather than Aβ plaques, is the primary cause of neuronal hyperexcitability [24]. For instance, Aβ1-42 (the most toxic form of soluble Aβ peptides) was to increase neuronal excitability through selective inhibition of K+ currents [26]. It was described that under the influence of Aβ, AD patients experience impaired neuronal and glial glutamate reuptake, leading to excitotoxicity. Similarly, glutamate excitotoxicity is also exacerbated by the effect of Aβ on the function of the N-methyl-D-aspartate receptor (NMDA-R) [27]. It was suggested that activation of cholinergic receptors and Ca^{2+} channels under the influence of Aβ may cause early subclinical epileptic activity preceding the development of clinical Alzheimer's disease [28]. Furthermore, it was shown that beta-secretase 1 (BACE1 is a key protein involved in Aβ formation) cleaves the β2 and β4 subunits of the voltage-gated Na^+ channel [24]. Cleavage of β2 alters transcription and receptor expression on the cell surface [21]; cleavage of β4 significantly increases intracellular

Table. Prevalence of subclinical epileptiform activity in patients with Alzheimer's disease

No	Literature reference	Cognitive impairment severity	People amount	SEA prevalence, %	Epileptiform Activity Index (EAI)	SEA localization	EEG type
1	Brunetti et al. [13]	AD MCI CG	50 50 50	AD – 6.38; MCI – 11.63; CG – 4.43	0.015–0.025/ hour	No data available	LTVEM + PSG + MEG
2	Vossel et al. [14]	AD + MCI	113	6	No data available	No data available	routine EEG
3	Liedorp et al. [15]	AD MCI other dementias	510 225 193	2 AD; 2 MCI; 1 other dementia	No data available	No data available	30-min EEG
4	Vossel et al. [16]	AD CG	33 19	42.4 AD; 10.5 CG	0.03–5.18/ hour	9.9% wakefulness; 25.7% N1, 64.4% N2-N3; 43% left temple; 29% left central area; 14% right frontal area; 14% bifrontotemporally	Nighttime PSG + MEG
5	Horvath et al. [17]	AD	42	28	No data available	No data available	24-h EEG
6	Horvath et al. [18]	AD CG	52 20	54 AD; 25 CG	0.29–6.68/ hour	8% wakefulness; 23% N1, 21% N2, 34% N3; 4% REM; 52% left temple; 22% right temple; 26% bitemporally; 3% biparietal; 3% right frontal area; 9% bifrontal	24-h EEG
7	Lam et al. [19]	AD CG	41 43	22 AD; 4.7 CG	1.5–3/day	20% N1, 80% N2; 85.7% left temporal region; 28.6% bifrontal	24-h EEG
8	Babiloni et al. [20]	AD c MCI; MCI without AD	56 32	No data available AD + MCI; 41 MCI without AD	No data available	No data available	routine EEG
9	Nous et al. [11]	AD+ dementia; AD + MCI; AD preclinical stage	49	31 among all patients with AD; 50 in dementia; 27 in MCI; 25 on clinical stage	Number of spikes per 50 min: Prolonged EEG: 0,19 spikes/min; 50-min MEG: 64.5 spikes/min; High-Density EEG: 3 spikes/min	Fronto-temporal regions (more often on the left). Single cases: central region, bifrontotemporal, bitemporal, right parietal, right temporal, right frontal regions. More often, stages 1 and 2 of sleep	Prolonged EEG and/ or 50-min MEG and/ or 50-min High-Density EEG

Table compiled by the authors based on data from sources [11, 13–20]

Note: MCI — mild cognitive impairment; CG — healthy control group; SEA — subclinical epileptiform activity; EEG — electroencephalography; LTVEM — long-term video-EEG monitoring, MEG — magnetoencephalography; PSG — polysomnography; N1 — sleep stage 1; N2 — sleep stage 2; N3 — sleep stage 3; REM — rapid eye movement sleep.

Na^+ levels [26]. Both processes contribute to overall neuronal hyperexcitability, which may promote the development of epileptic seizures.

Both epilepsy and Alzheimer's disease involve neuroinflammation induced by Aβ, characterized by the induction of an immune response in the central nervous system (CNS) in reaction to the pathological process [29]. Inflammation in the CNS is primarily mediated by microglia, astrocytes, and oligodendrocytes [30]. Aβ-induced glial activation leads to the release of numerous pro-inflammatory cytokines (e.g., TNF-α, IL-6, or IL-1β), triggering generalized neuroinflammation. This process, in turn, promotes neurotoxic effects that ultimately result in neuronal hyperexcitability, exacerbating the neurodegenerative process [24]. It was also described that pro-inflammatory cytokines, such as IL-1β, increase neuronal hyperexcitability either by enhancing glutamate release from astrocytes and reducing its reuptake [31], or by up-regulating NMDA-R, which increases intracellular Ca^{2+} influx [32].

Tau protein plays a distinct role in epileptogenesis during AD, given that this protein is one of the key mediators of Aβ-induced epileptogenic mechanisms [33]. Tau protein contributes to neuronal excitotoxicity by increasing extracellular glutamate and causing NMDA-R dysfunction [34]. Tau protein is also associated with abnormal neuronal migration in the hippocampus—a brain structure closely linked to the development of epilepsy [35, 36]. Furthermore, animal models of epileptogenesis confirmed reduced activity of phosphatase 2A, leading to increased p-tau in epileptogenic brain regions [37].

The neurosteroid allopregnanolone was also linked to the development of Alzheimer's disease [38]. Some authors reported decreased levels of allopregnanolone in the plasma and brain, particularly in the prefrontal cortex, of patients with AD. Reduced allopregnanolone levels lead to diminished neuroprotection, activation of astrocytes and microglia, which in turn promotes the production of neurotoxic cytokines, chemokines, and reactive oxygen and nitrogen species. These mechanisms contribute to the progression of neurodegenerative disease and neuronal hyperexcitability [38].

The key components of the pathogenetic relationship between epileptiform activity and the neurodegenerative process are presented in the Figure below. Increased activity of the glutamatergic system in AD leads to

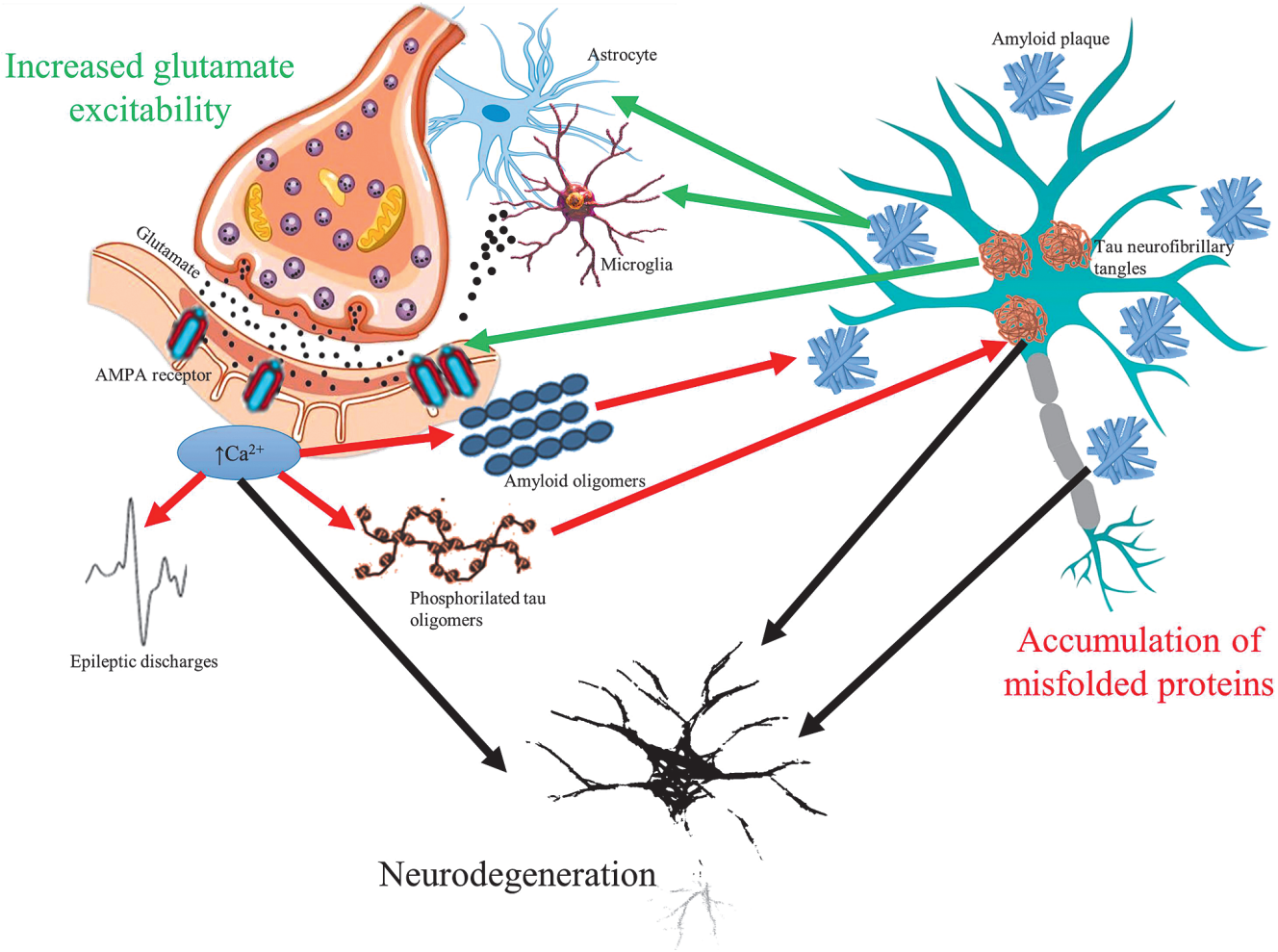

Figure prepared by the authors based on data from [23], CC BY license

Fig. Vicious cycle of glutamate-mediated hyperexcitability and accumulation of pathological proteins in cognitive impairment in Alzheimer's disease

elevated expression of AMPA receptors and mobilization of intracellular calcium. The rise in intracellular calcium levels results in the release of amyloid oligomers into the extracellular space and enhanced phosphorylation of tau oligomers (red arrows). Increased neuronal excitation, manifested as epileptic discharges, is also a consequence of glutamate-mediated hyperexcitability. On the other hand, the accumulation of amyloid plaques and tau neurofibrils alters the expression of glutamate receptors and triggers excessive glutamate release from microglial cells and astrocytes (green arrows). This bidirectional pathological interaction can lead to progressive neurodegeneration (black arrows), which is typically accompanied by cognitive impairment [23].

Pathological remodeling of hippocampo-cortical connections is also considered to play a major role in the presence of epileptiform activity. As a result of epileptiform activity, local intrahippocampal connections are strengthened, while the strength and number of long-range connections are reduced. This remodeling of neural networks leads to relative isolation of the hippocampus from the cortex, impairing the functioning of hippocampo-cortical circuits [23].

Furthermore, the presence of epileptiform activity disrupts physiological sleep patterns and impairs the memory consolidation process. Thalamic sleep spindles at a frequency of 12–16 Hz are crucial for memory formation, synchronizing hippocampal activity with cortical neurons. Slow waves associated with cortical sleep provide the highest degree of synchronization, promoting the activation of hippocampal activity and thalamic sleep spindles. Epileptiform activity contributes to:

- transformation of hippocampal activity;
- disorganization of sleep spindle architecture;
- reduction of cortical slow waves due to cortical hyperpolarization.

In combination, these changes reduce the efficiency of memory consolidation [23].

Treatment of subclinical epileptiform activity as an alternative approach to AD therapy

Given the existing concept of SEA potentiating the pathophysiological mechanisms that contribute to the progression of cognitive impairment in AD, some authors propose therapeutic approaches for treating AD patients with SEA, such as prescribing antiseizure medication (ASM). There is a wide range of ASMs available; however, due to the negative effects of most of them on cognitive functions and memory, the choice of ASM in such patients is limited.

According to numerous studies on the effects of ASMs on cognitive functions in patients with epilepsy, some drugs exhibit a so-called “pro-cognitive” effect. Levetiracetam is one example of such drugs. Due to the potentially beneficial effects of levetiracetam on cognitive functions, most studies aimed at treating SEA and epilepsy in AD patients focus on this particular medication [14, 39–44]. Experiments showed that levetiracetam modulates neuronal hyperexcitability, reduces the number of amyloid plaques, and regulates neurotrophic factors [39, 45]. It is known that in AD patients with epileptiform activity, cognitive functions deteriorate faster than in those without such activity. For instance, Vossel et al. studied the effects of levetiracetam on various domains of cognitive function in a group of 34 participants with AD. The analysis showed that in the group of patients with seizures or SEA, the use of levetiracetam led to positive dynamics in tests of executive function and visuospatial memory [46].

Lamotrigine, which has no negative effect on cognitive functions, may also be considered for use in patients with AD and SEA [12, 45, 47–50]. Lamotrigine prevents the accumulation of extracellular β-amyloid, suppresses glutamate excitotoxicity, thereby exerting neuroprotective properties [51, 52]. A study by Tekin et al. of AD patients without epilepsy showed that the use of lamotrigine at a dose of 300 mg/day for eight weeks had a positive effect on cognitive indicators (in performing tasks on recognition and naming of objects and matching names with objects) and mood [52]. However, there are currently no clear clinical guidelines for prescribing antiseizure therapy to AD patients with SEA having no seizures, which requires further study.

CONCLUSION

The conducted review indicates the high clinical significance of performing electroencephalography and detecting epileptiform activity in patients with Alzheimer’s disease due to its potential negative impact on the progression of cognitive impairment and increased risk of epileptic seizures in such patients. The frequency of SEA in AD patients can vary (2–54%) depending on the duration of EEG recording, sleep inclusion, and the use of additional techniques (MEG, *foramen ovale* electrodes). Most literature data emphasize a higher incidence of SEA in AD patients compared to those with other types of dementia or healthy individuals of the corresponding age. Pathophysiological mechanisms highlight common etiopathogenetic links in the progression of AD and the formation of neuronal hyperexcitability, which is associated with the appearance of epileptiform activity on EEG. The use of ASM for SEA therapy may become a new treatment strategy for AD patients, not only as a means of preventing epileptic seizures but also in the treatment of cognitive impairment. However, the advisability of treating subclinical epileptiform activity in Alzheimer’s disease patients remains a subject for further investigation.

References

1. Levin OS. *Diagnostics and treatment of cognitive disorders and dementia in clinical practice.* 3rd ed. Moscow: MEDpress-Inform; 2023 (In Russ).
2. Prince M, Wimo A, Guerchet M, Ali GC, Wu YuT, Prina M. *World Alzheimer Report, 2015. The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends*. London: ADI; 2015.
3. Belousov YB, Zyryanov SK, Belousov DY, Beketov AS. Clinical and economic aspects of Alzheimer's disease therapy in Russia. *Good Clinical Practice.* 2018;1:3–28 (In Russ.).
4. Hurd MD, Martorell P, Delavande A, Mullen KJ, Langa KM. Monetary costs of dementia in the United States. *The New England Journal of Medicine.* 2013;68(14):1326–34. https://doi.org/doi:10.1056/nejmsa1204629
5. Koberskaya NN. Alzheimer's disease. *Neurology, Neuropsychiatry, Psychosomatics*. 2019;11(3S):52–60 (In Russ.). https://doi.org/10.14412/2074-2711-2019-3S-52-60
6. Babiloni C, Carducci F, Lizio R, Vecchio F, Baglieri A, Bernardini S, et al. Resting state cortical electroencephalographic rhythms are related to gray matter volume in subjects with mild cognitive impairment and Alzheimer's disease. *Human Brain Mapping*. 2013;34(6):1427–46. https://doi.org/doi:10.1002/hbm.22005
7. Kang JQ. Epileptic mechanisms shared by Alzheimer's disease: Viewed via the unique lens of genetic epilepsy. *International Journal of Molecular Sciences*. 2021;22(13):7133. https://doi.org/10.3390/ijms22137133
8. Musaeus CS, Engedal K, Høgh P, Jelic V, Mørup M, Nail M, et al. EEG theta power is an early marker of cognitive decline in dementia due to Alzheimer's disease. *Journal of Alzheimer's Disease*. 2018;64(4):1359–71. https://doi.org/10.3233/jad-180300
9. Cretin B, Bousiges O, Hautecloque G, Philippi N, Blanc F, Dibitonto L, et al. CSF in epileptic prodromal Alzheimer's disease: No diagnostic contribution but a pathophysiological one. *Frontiers in Neurology*. 2021;12:623777. https://doi.org/10.3389/fneur.2021.623777
10. Csernus EA, Werber T, Kamondi A, Horvath AA. The significance of subclinical epileptiform activity in Alzheimer's disease: A review. *Frontiers in Neurology*. 2022;13:856500. https://doi.org/10.3389/fneur.2022.856500
11. Nous A, Seynaeve L, Feys O, Wens V, de Tiege X, van Mierlo P, et al. Subclinical epileptiform activity in the Alzheimer continuum: association with disease, cognition and detection method. *Alzheimer's Research and Therapy.* 2024;16(1):19. https://doi.org/10.1186/s13195-023-01373-9
12. Mendez MF, Lim GTH. Seizures in elderly patients with dementia. *Drugs and Aging.* 2003;20(11):791–803. https://doi.org/10.2165/00002512-200320110-00001
13. Brunetti V, D'Atri A, Della Marca G, Vollono C, Marra C, Vita MG, et al. Subclinical epileptiform activity during sleep in Alzheimer's disease and mild cognitive impairment. *Clinical Neurophysiology*. 2020;131(5):1011–8. https://doi.org/10.1016/j.clinph.2020.02.015
14. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Nassan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurology*. 2013;70(9):1158–66. https://doi.org/10.1001/jamaneurol.2013.136
15. Liedorp M, Stam CJ, van der Flier WM, Pijenburg YAL, Scheltens P. Prevalence and clinical significance of epileptiform EEG discharges in a large memory clinic cohort. *Dementia and Geriatric Cognitive Disorders.* 2010;29(5):432–7. https://doi.org/10.1159/000278620
16. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Annals of Neurology.* 2016;80(6):858–70. https://doi.org/10.1002/ana.24794
17. Horváth A, Szűcs A, Hidasi Z, Csukly G, Barcs G, Kamondi A. Prevalence, semiology, and risk factors of epilepsy in Alzheimer's disease: An ambulatory EEG study. *Journal of Alzheimer's Disease.* 2018;63(3):1045–54. https://doi.org/10.3233/JAD-170925
18. Horvath AA, Papp A, Zsuffa J, Szucs A, Luckl J, Radai F, et al. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: A long-term EEG study. *Clinical Neurophysiology.* 2021;132(8):1982–9. https://doi.org/10.1016/j.clinph.2021.03.050
19. Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology*. 2020;95(16):e2259–70. https://doi.org/10.1212/WNL.0000000000010612
20. Babiloni C, Noce G, Di Bonaventura C, Lizio R, Pascarelli MT, Tucci F, et al. Abnormalities of cortical sources of resting state delta electroencephalographic rhythms ere related to epileptiform activity in patients with amnesic mild cognitive impairment not due to Alzheimer's disease. *Frontiers in Neurology.* 2020;11:514136. https://doi.org/10.3389/fneur.2020.514136
21. Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nature Medicine*. 2017;23(6):678–80. https://doi.org/10.1038/nm.4330
22. Ung H, Cazares C, Nanivadekar A, Kini L, Wagenaar J, Becker D, et al. Interictal epileptiform activity outside the seizure onset zone impacts cognition. *Brain*. 2017;140(8):2157–68. https://doi.org/10.1093/brain/awx143
23. Horvath AA, Csernus EA, Lality S, Kaminski RM, Kamondi A. Inhibiting epileptiform activity in cognitive disorders: possibilities for a novel therapeutic approach. *Frontiers in Neuroscience.* 2020;14:557416. https://doi.org/10.3389/fnins.2020.557416
24. Giorgi FS, Saccaro LF, Busceti CL, Biagioni F, Fornai F. Epilepsy and Alzheimer's disease: potential mechanisms for an association. *Brain Research Bulletin*. 2020;160:107–20. https://doi.org/10.1016/j.brainresbull.2020.04.009
25. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *The Lancet Neurology*. 2017;16(4):311–22. https://doi.org/10.1016/S1474-4422(17)30044-3
26. Ye CP, Selkoe DJ, Hartley DM. Protofibrils of amyloid β-protein inhibit specific K^+ currents in neocortical cultures. *Neurobiology of Disease*. 2003;13(3):177–90. https://doi.org/10.1016/s0969-9961(03)00068-8
27. Lei M, Xu H, Li Z, Wang Z, O'Malley TT, Zhang D, et al. Soluble Aβ oligomers impair hippocampal LTP by disrupting glutamatergic/GABAergic balance. *Neurobiology of Disease.* 2016;85:111–21. https://doi.org/10.1016/j.nbd.2015.10.019
28. Kam K, Duffy ÁM, Moretto J, LaFrancois J, Scharfman HE. Interictal spikes during sleep are an early defect in the Tg2576 mouse model of β-amyloid neuropathology. *Scientific Reports*. 2016;6:20119. https://doi.org/10.1038/srep20119

29. Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimer's and Dementia.* 2016;12(6):719–32. https://doi.org/10.1016/j.jalz.2016.02.010
30. Regen F, Hellmann-Regen J, Costantini E, Reale M. Neuroinflammation and Alzheimer's disease: implications for microglial activation. *Current Alzheimer Research*. 2017;14(11):1140–8. https://doi.org/10.2174/1567205014666170203141717
31. Vezzani A, Friedman A, Dingledine RJ. The role of inflammation in epileptogenesis. *Neuropharmacology*. 2013;69:16–24. https://doi.org/10.1016/j.neuropharm.2012.04.004
32. Postnikova TY, Zubareva OE, Kovalenko AA, Kim KK, Magazinik LG, Zaitsev AV. Status epilepticus impairs synaptic plasticity in rat hippocampus and is followed by changes in expression of NMDA receptors. *Biochemistry.* 2017;82(3):282–90. https://doi.org/10.1134/S0006297917030063
33. Roberson ED, Scearce-Levie K, Palop JJ, Fengrong Y, Cheng IH, Wu T, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science.* 2007;316(5825):750–4. https://doi.org/10.1126/science.1141736
34. Decker JM, Krüger L, Sydow A, Dennissen FJA, Siskova Z, Mandelkow E, et al. The Tau/A152T mutation, a risk factor for frontotemporal-spectrum disorders, leads to NR2B receptor-mediated excitotoxicity. *EMBO Reports.* 2016;17(4):552–69. https://doi.org/10.15252/embr.201541439
35. Tian FF, Zeng C, Ma YF, Guo TH, Chen JM, Cai XF, et al. Potential roles of Cdk5/p35 and tau protein in hippocampal mossy fiber sprouting in the PTZ kindling model. *Clinical Laboratory*. 2010;56(3–4):127–36.
36. Thom M, Liu JYW, Thompson P, Pladke R, Narkiewicz M, Martinian L, et al. Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study. *Brain*. 2011;134(10):2969–81. https://doi.org/10.1093/brain/awr209
37. Liu SJ, Zheng P, Wright DK, Deszi G, Braine E, Nguen T, et al. Sodium selenate retards epileptogenesis in acquired epilepsy models reversing changes in protein phosphatase 2A and hyperphosphorylated tau. *Brain*. 2016;139(7):1919–38. https://doi.org/10.1093/brain/aww116
38. Luchetti S, Huitinga I, Swaab DF. Neurosteroid and GABA-A receptor alterations in Alzheimer's disease, Parkinson's disease and multiple sclerosis. *Neuroscience.* 2011;191:6–21. https://doi.org/10.1016/j.neuroscience.2011.04.010
39. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *PNAS.* 2012;109(42):E2895–903. https://doi.org/10.1073/pnas.1121081109
40. Corbett BF, You JC, Zhang X, Pyfer MS, Tosi U, Iaskone DM, et al. ΔFosB regulates gene expression and cognitive dysfunction in a mouse model of Alzheimer's disease. *Cell Reports*. 2017;20(2):344355. https://doi.org/10.1016/j.celrep.2017.06.040
41. Shi JQ, Wang BR, Tian YY, Xu J, Gao L, Zhao SL, et al. Antiepileptics topiramate and levetiracetam alleviate behavioral deficits and reduce neuropathology in APPswe/PS1dE9 transgenic mice. *CNS Neuroscience and Therapeutics.* 2013;19(11):871–81. https://doi.org/10.1111/cns.12144
42. Nygaard HB, Kaufman AC, Sekine-Konno T, Huh LL, Going H, Feldman SJ, et al. Brivaracetam, but not ethosuximide, reverses memory impairments in an Alzheimer's disease mouse model. *Alzheimer's Research and Therapy.* 2015;7(1):25. https://doi.org/10.1186/s13195-015-0110-9
43. Fu CH, Iascone DM, Petrof I, Hazra A, Zhang X, Pyfer MS, et al. Early seizure activity accelerates depletion of hippocampal neural stem cells and impairs spatial discrimination in an Alzheimer's disease model. *Cell Reports*. 2019;27(13):3741–51. https://doi.org/10.1016/j.celrep.2019.05.101
44. Cumbo E, Ligori LD. Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. *Epilepsy and Behavior.* 2010;17(4):461–6. https://doi.org/10.1016/j.yebeh.2010.01.015
45. Horváth A, Szűcs A, Barcs G, Noebels JL, Kamondi A. Epileptic seizures in Alzheimer disease: A review. *Alzheimer Disease and Associated Disorders.* 2016;30(2):186–92. https://doi.org/10.1097/WAD.0000000000000134
46. Vossel K, Ranasinghe KG, Beagle AJ, La A, Pook KA, Castro M, et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: A randomized clinical trial. *JAMA Neurology.* 2021;78(11):1345–54. https://doi.org/10.1001/jamaneurol.2021.3310
47. Nicastro N, Assal F, Seeck M. From here to epilepsy: the risk of seizure in patients with Alzheimer's disease. *Epileptic Disorders.* 2016;18(1):1–12. https://doi.org/10.1684/epd.2016.0808
48. Rowan AJ, Ramsay RE, Collins JF, Pryor F, Boardman KD, Uthman BM, et al. New onset geriatric epilepsy: A randomized study of gabapentin, lamotrigine, and carbamazepine. *Neurology*. 2005;64(11):1868–73. https://doi.org/10.1212/01.wnl.0000167384.68207.3e
49 Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: Causes and clinical relevance. *The Lancet Neurology.* 2017;16(4):311–22. https://doi.org/10.1016/s1474-4422(17)30044-3
50. Javed A, Cohen B, Detyniecki K, Hirsch LJ, Legge A, Chen B, et al. Rates and predictors of patient-reported cognitive side effects of antiepileptic drugs: An extended follow-up. *Seizure.* 2015;29:34–40. https://doi.org/10.1016/j.seizure.2015.03.013
51. Rizzello E, Pimpinella D, Pignataro A, Titta G, Merenda E, Saviana M, et al. Lamotrigine rescues neuronal alterations and prevents seizure-induced memory decline in an Alzheimer's disease mouse model. *Neurobiology of Disease.* 2023;181:106106. https://doi.org/10.1016/j.nbd.2023.106106
52. Tekin S, Aykut-Bingöl C, Tanridağ T, Aktan S. Antiglutamatergic therapy in Alzheimer's disease — effects of lamotrigine. Short communication. *Journal of Neural Transmission.* 1998;105(2–3):295–303. https://doi.org/10.1007/s007020050059

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DETERMINATION OF PREDICTORS OF ADVERSE DISEASE OUTCOME IN PATIENTS WITH COVID-19 BASED ON HEMOSTASIS SYSTEM ANALYSIS

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Introduction. Severe complications of the novel coronavirus infection (COVID-19) include arterial or venous thromboses, which not only complicate the disease course but also increase mortality. The development of hypercoagulability, which precedes the occurrence of thrombosis, is associated with a significant activation of the hemostasis system, as well as the appearance of microparticles in circulation. These microparticles, generated by activated blood cells, enhance the procoagulant orientation of hemostasis. In this regard, assessment of the prognostic value of changes in hemostasis system parameters associated with the progression and outcome of COVID-19 represents a relevant research task.
Objective. To identify predictors of adverse outcomes of the novel coronavirus infection based on the assessment of parameters characterizing the state of the hemostasis system.
Materials and methods. A total of 163 patients (78 males and 85 females, aged 35–90 years, median age 69 years) were examined during the acute phase of the disease with severe and moderate severity. Depending on the disease outcome, the patients were divided into two groups: the group of survivors (n = 120) and the group of the deceased (n = 43). A study of plasma hemostasis parameters was conducted, including Quick's prothrombin test, fibrinogen concentration, activated partial thromboplastin time, factor VIII activity, ristocetin cofactor activity, von Willebrand factor content, protein C activity, antithrombin, and free protein S. In addition, the characteristics of microparticles were studied. Statistical processing of the results was performed using the Statistica 12.0 software package.
Results. In patients with adverse disease outcomes, a significant decrease in Quick's prothrombin time (PT) and antithrombin activity was observed, along with an increase in von Willebrand factor activity, D-dimer concentration, and platelet microparticle count. The analysis of sensitivity and specificity of these parameters allowed Quick's PT less than 70% (sensitivity and specificity were 70% and 74.3%, respectively), D-dimer level more than 800 ng/ml (sensitivity and specificity — 72% and 75.2%, respectively), and platelet MP count more than 3.22% (sensitivity and specificity — 77.8% and 72.7%, respectively) to be considered as threshold values associated with lethal outcome from COVID-19.
Conclusions. Based on the conducted ROC analysis, predictive models for the risk of adverse outcomes of COVID-19 associated with changes in hemostasis system parameters were obtained. The parameters of D-dimer concentration, Quick's prothrombin time, and platelet microparticle count can be used as laboratory predictors of unfavorable disease progression.

Keywords: adverse outcome; COVID-19; hypercoagulation; Quick's prothrombin test; D-dimer; platelet microparticles

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ОПРЕДЕЛЕНИЕ ПРЕДИКТОРОВ НЕБЛАГОПРИЯТНОГО ИСХОДА ЗАБОЛЕВАНИЯ У ПАЦИЕНТОВ С COVID-19 НА ОСНОВАНИИ ИССЛЕДОВАНИЯ СИСТЕМЫ ГЕМОСТАЗА

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Введение. Среди тяжелых осложнений новой коронавирусной инфекции (COVID-19) выделяют артериальные или венозные тромбозы, которые приводят не только к более тяжелому течению заболевания, но и к увеличению летальности. Развитие гиперкоагуляции, предшествующее реализации тромбоза, обусловлено выраженной активацией системы гемостаза, а также появлением в циркуляции микрочастиц (МЧ), которые генерируются активированными клетками крови и увеличивают прокоагулянтную направленность гемостаза. В связи с этим оценка прогностического значения изменений показателей системы гемостаза, связанных с течением и исходом COVID-19, представляет большой интерес.
Цель. Выявить предикторы неблагоприятного исхода новой коронавирусной инфекции на основе оценки параметров, характеризующих состояние системы гемостаза.
Материалы и методы. Обследовано 163 пациента (78 мужчин и 85 женщин, возраст которых колебался от 35 до 90 лет, медиана возраста — 69 лет) в остром периоде с тяжелым и среднетяжелым течением заболевания. В зависимости от исхода заболевания

пациенты были разделены на две группы: группа «выжившие пациенты» (*n* = 120); группа «умершие пациенты» (*n* = 43). Проведено исследование показателей плазменного гемостаза (протромбинового теста по Квику, концентрации фибриногена, активированного парциального тромбопластинового времени, активности фактора VIII, ристоцетин-кофакторной активности и содержания фактора Виллебранда, активности протеина С, антитромбина, уровня свободного протеина S), а также оценка характеристик МЧ. Статистическую обработку полученных результатов выполняли с помощью пакета программного обеспечения Statistica 12.0.

Результаты. У пациентов с неблагоприятным исходом заболевания получено значимое снижение протромбинового теста (ПТ) по Квику и активности антитромбина, повышение активности фактора Виллебранда, концентрации D-димера и количества тромбоцитарных МЧ. Проведенный анализ чувствительности и специфичности данных параметров позволил рассматривать ПТ по Квику менее 70% (чувствительность и специфичность составили 70 и 74,3% соответственно), уровень D-димера более 800 нг/мл (чувствительность и специфичность — 72 и 75,2% соответственно) и количество тромбоцитарных МЧ более 3,22% (чувствительность и специфичность — 77,8 и 72,7% соответственно) в качестве пороговых значений, ассоциированных с летальным исходом от COVID-19.

Выводы. На основании проведенного ROC-анализа получены прогностические модели риска возникновения неблагоприятного исхода COVID-19, сопряженные с изменениями параметров системы гемостаза: концентрации D-димера, ПТ по Квику и количества тромбоцитарных МЧ, которые могут быть использованы в качестве лабораторных предикторов неблагоприятного течения заболевания.

Ключевые слова: неблагоприятный исход; COVID-19; гиперкоагуляция; протромбиновый тест по Квику; D-димер; тромбоцитарные микрочастицы

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Соответствие принципам этики: исследование проведено в соответствии с Хельсинкской декларацией «Этические принципы медицинских исследований с участием человека в качестве испытуемого» от 1964 г. (ред. 2008 г.). Одобрено локальным этическим комитетом ФГБУ «Российский научно-исследовательский институт гематологии и трансфузиологии Федерального медико-биологического агентства» (протокол № 14 от 20.03.2025). Всеми пациентами подписано добровольное информированное согласие на исследование.

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INTRODUCTION

It has been established that the novel coronavirus infection (COVID-19) is associated with the development of endothelial dysfunction, activation of blood cells with the formation of blood plasma microparticles, plasma fibrinolytic failure, and the development of a cytokine storm syndrome [1, 2]. Such pathological changes lead to a procoagulant shift in the hemostatic system, which may be associated with the development of hypercoagulability followed by thrombosis in the microvasculature, distress syndrome, and multiple organ failure. Thromboembolic complications are among the leading causes of increased mortality in patients with COVID-19.

Studies have shown that the frequency of thrombotic complications reaches 18% in patients treated in intensive care units [3, 4]. During COVID-19, activation of the hemostatic system occurs, affecting both its plasma and cellular components, leading to a prothrombotic state [5–8]. Microparticles derived from various blood cells and capable of participating in a range of biological reactions may play a significant role in these procoagulant changes. Plasma microparticles are phospholipid microvesicles ranging 0.1–1 μm in size. They are surrounded by a cell membrane, lack a nucleus, and vary significantly in the composition of antigenic determinants on their surface, depending on the mechanism of their formation and the nature of the stimulating influence. Due to the negatively charged phospholipids and tissue factor localized on their surface, MPs actively participate in hemostatic reactions, which may be a significant factor in the development of thrombotic complications in various pathologies, including COVID-19 [9–11].

The use of various laboratory and instrumental studies to identify predictors of adverse outcomes and progression of the novel coronavirus infection is of great interest. Thus, determination of the SARS-CoV-2 viral load and assessment of lung CT results upon hospitalization using artificial intelligence have shown good prognostic value; however, these methods are not widely available for clinical use [12, 13]. Certain informativeness is offered by the level of lymphopenia and changes in lymphocyte subpopulations, as well as such indicators as C-reactive protein, procalcitonin, and ferritin, which are nonspecific markers of inflammation [14–17]. Given the characteristic changes in the blood coagulation system accompanying COVID-19, the search for such markers is also conducted among hemostatic parameters. A number of researchers have noted a correlation between high D-dimer levels and mortality, although its threshold prognostic values vary widely [18–20].

The selection of parameters characterizing the state of plasma hemostasis and the degree of blood cell activation, which can be used for prognostic assessment of

disease severity and outcome, will contribute to a more rational management of patients with COVID-19.

This study is aimed at identifying predictors of adverse outcomes of the novel coronavirus infection based on the assessment of parameters characterizing the state of the hemostasis system.

MATERIALS AND METHODS

The study group included 163 patients (78 males and 85 females, aged 35–90 years, median age 69 years) with severe or moderate forms of COVID-19. All the patients were treated in intensive care units. The inclusion criteria were the age over 18 years old and confirmed novel coronavirus infection with a positive laboratory test for SARS-CoV-2 RNA. The exclusion criteria were the age under 18 years, history or presence of oncological disease, HIV infection, hepatitis B and C, liver pathology with impaired function, kidney disease with altered glomerular filtration rate, and regular use of any anticoagulant drugs prior to the onset of the disease.

The severity of the disease was determined by the degree of lung involvement, which exceeded 25%, as well as patient comorbidities. Among the examined patients, 120 (74%) recovered, while 43 (26%) had an adverse (fatal) outcome. Depending on the disease outcome, patients were divided into two groups for the assessment of hemostasis parameters and microparticle (MP) characteristics: the group of survivors (n = 120) and the group of deceased patients (n = 43).

Blood samples for the study were collected upon patients' admission to the hospital, prior to the initiation of specific therapy and anticoagulant prophylaxis, using a vacuum system with Vacutest vacuum tubes containing 3.2% sodium citrate as an anticoagulant.

The following parameters characterizing the state of plasma hemostasis were evaluated: Quick's prothrombin time (PT), fibrinogen concentration (Fg), activated partial thromboplastin time (APTT), factor VIII activity (f.VIII), ristocetin cofactor activity and von Willebrand factor content (vWF:RCof and vWF:Ag, respectively), protein C activity (PC) and antithrombin (AT), as well as free protein S level (PS). Reagents from HemosIL (Instrumentation Laboratory, USA) were used, and all studies were performed in accordance with the manufacturer's instructions for reagents and equipment. The listed parameters were determined using automated coagulometers of the ACL series, including ACL Top 300 CTS and ACL Elite Pro (Automated Coagulation Laboratory, Instrumentation Laboratory, USA).

For the analysis of microparticle characteristics using flow cytometry, platelet-poor plasma was centrifuged using a ThermoFisherScientific centrifuge (Germany) at 22°C for 30 min at 14,000 g. After high-speed centrifugation, the supernatant was aspirated, and the pellet was collected and resuspended by adding 100 µL of phosphate-buffered saline (PBS). The resulting microparticle suspension was used for further analysis. To determine the quantity and origin of microparticles, a laser flow cytometer Cytoflex (Beckman Coulter, USA) was employed using fluorescently labeled antibodies against surface markers of cells as follows: platelet-derived (CD41), leukocyte-derived (CD45), and endothelial-derived (CD144).

Statistical analysis of the results obtained was performed using the Statistica 12.0 software (StatSoft Inc., USA). Asymmetrical data distribution was identified using the Shapiro–Wilk test; the results are presented as median (M_e) and interquartile [25–75 percentile] range [Q_1–Q_3]. Comparison between the two groups was conducted using the non-parametric Mann–Whitney U-test. To identify the threshold values of variables associated with adverse outcomes of COVID-19, ROC analysis was performed with the construction of ROC curves. The quantitative interpretation of ROC curves was assessed using the Area Under the Curve (AUC) metric, which represents the area bounded by the ROC curve and the axis of false positive rates. An AUC value below 0.5 indicated the unsuitability of the selected classification method, while an AUC above 0.7 characterized the high predictive power of the constructed model. The Youden index was used as a criterion for determining the optimal threshold point or cutoff value along the ROC curve, allowing evaluation of the difference between the proportion of true positive results (sensitivity) and the proportion of false positive results (specificity) to select the optimal threshold value. The critical level of statistical significance was set at 0.05.

RESULTS

The results obtained by comparing the plasma hemostasis parameters of the examined patients, depending on the disease outcome, are presented in Table 1.

The results presented in Table 1 indicate significant differences between the two groups of examined patients. Thus, an adverse disease outcome was associated with a 1.4-fold increase in von Willebrand factor activity (340.0 [260.1–420.0]; p = 0.01), a 1.2-fold decrease in Quick's prothrombin time (65.3 [51.0–73.9]; p = 0.00), and a reduction in antithrombin activity to 85.3 [71.0–97.5]; p = 0.034 when compared to the group of survivors. The most significant differences involved the concentration of D-dimer, which was nearly four times higher in deceased patients (1670.0 [715.0–4334.5]; $p \leq 0.0001$) relative to levels in patients with a favorable disease outcome. These findings are consistent with data obtained by other researchers who have also identified a significant increase in D-dimer levels as a marker of adverse progression and outcome of the novel coronavirus infection [21, 22].

Additionally, we conducted an analysis of plasma microparticle characteristics in the examined patients based on disease outcome. The obtained results are presented in Table 2.

Table 1. Parameters characterizing the plasma hemostasis status of COVID-19 patients

Parameters	Survivors (*n* = 120)	Deceased (*n* = 43)	Level of statistical significance*, *p*
APTT	0.88 [0.82–0.97]	0.88 [0.82–1.08]	0.407
PT, %	79.0 [70.2–85.1]	65.3 [51.0–73.9]	0.000
FG, g/L	5.4 [4.1–6.9]	5.5 [3.6–8.0]	0.931
F.VIII, %	112.4 [85.8–165.5]	150.0 [80.3–217.2]	0.08
vWF:RCo, %	250.5 [180.0–350.1]	340.0 [260.1–420.0]	0.01
vWF:Ag, %	219.6 [198.3–321.2]	340.0 [260.1–420.0]	0.08
D-dimer, ng/mL	387.0 [220.0–724.5]	1670.0 [715.0–4334.5]	<0.0001
AT, %	97.7 [84.3–105.0]	85.3 [71.0–97.5]	0.034
PC, %	97.0 [79.7–117.3]	88.0 [67.2–102.0]	0.185
PS, %	75.2 [56.5–90.0]	62.7 [48.2–86.3]	0.138

Table compiled by the authors based on original data

Note: * — comparison was performed between groups of COVID-19 patients; APTT — activated partial thromboplastin time, PT — Quick's prothrombin time, FG — fibrinogen concentration, f.VIII — factor VIII activity, vWF:RCo — ristocetin cofactor activity, vWF:Ag — von Willebrand factor antigen, AT — antithrombin activity, PC — protein C activity, PS — free protein S.

Table 2. Characterization of microparticles in COVID-19 patients

Cellular Marker	Survivors (*n* = 15)	Deceased (*n* = 9)	Level of statistical significance*, *p*
$CD41^+$ (% of events)	2.22 [1.385–3.25]	4.27 [3.48–4.61]	0.025
$CD144^+$ (% of events)	0.03 [0.02–0.03]	0.05 [0.03–0.06]	0.8

Table compiled by the authors based on original data

Note: * — comparison was performed between the study groups of COVID-19 patients.

The data presented in Table 2 indicate a significant increase in platelet-derived microparticles in patients with adverse disease outcomes compared to survivors. No significant differences were observed between the two groups in the number of endothelial-derived MPs. Leukocyte-derived microparticles were detected in negligible quantities, which precluded robust statistical analysis.

For further analysis, results from tests with parameters that significantly differed between patient groups based on disease outcome were selected, namely:

- D-dimer concentration,
- von Willebrand factor (vWF) activity,
- antithrombin (AT) activity,
- Quick's prothrombin time (PT),
- platelet-derived MP count.

ROC analysis of vWF activity and antithrombin activity did not yield high-quality predictive models for adverse outcomes of COVID-19. Conversely, ROC analysis for parameters such as D-dimer, Quick's PT, and platelet-derived MP count — which are directly associated with adverse COVID-19 outcomes — demonstrated models with a high predictive power:

- AUC for D-dimer 0.787;
- AUC for Quick's PT 0.747;
- AUC for platelet-derived MPs 0.798.

The results allowed the determination of threshold values for these parameters, indicating a high probability of lethal outcome. For D-dimer levels, the highest Youden index (47.2), corresponding to a sensitivity of 72% and specificity of 75.2%, was achieved at a threshold value of **800 ng/mL** (Fig. 1).

For the indicator of Quick's prothrombin time (PT), the highest Youden index of 44.3, corresponding to a model sensitivity of 70% and specificity of 74.3%, was achieved at a threshold value of 70% (Fig. 2).

For the platelet-derived microparticle count, the highest Youden index of 50.5, corresponding to a sensitivity of 77.8% and specificity of 72.7%, was achieved at a threshold value of 3.22% of events (Fig. 3).

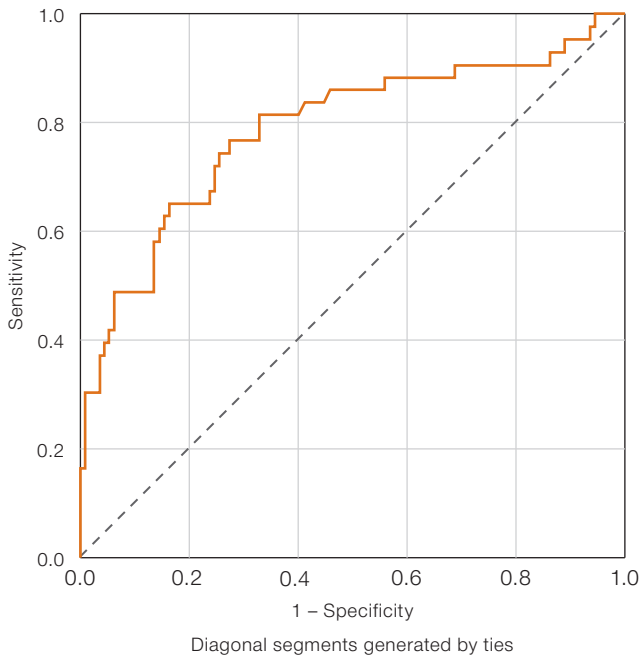

Figure prepared by the authors based on original data

Fig. 1. ROC curves for evaluating the predictive model of elevated D-dimer levels and adverse outcomes in COVID-19

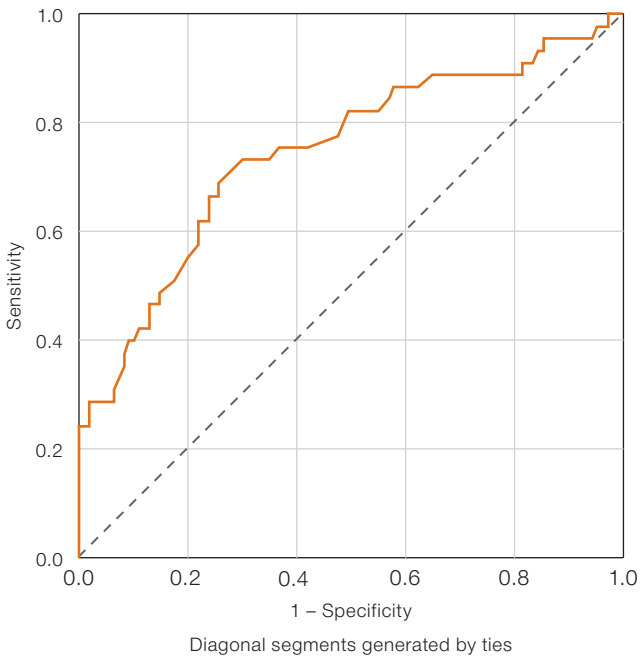

Figure prepared by the authors based on original data

Fig. 2. ROC curves for evaluating the predictive model of Quick's prothrombin test (PT) reduction and adverse outcome in COVID-19

Thus, the analysis of sensitivity and specificity of the selected parameters allows us to consider the following markers as predictors of an adverse outcome in COVID-19:

- Quick's prothrombin time (PT) below 70%,
- D-dimer level above 800 ng/mL,
- Platelet-derived microparticle (MP) count above 3.22% of events.

CONCLUSION

Disturbances in the hemostatic system play a key role in the pathogenesis of COVID-19 complications. Prothrombotic changes lead to the development of thrombotic processes in vessels of various types and calibers, thus aggravating the disease prognosis. Our study revealed that a number of parameters characterizing the state of the hemostasis system, namely D-dimer concentration and Quick's prothrombin time (PT), can be used as laboratory predictors of lethal disease outcomes. Given the limited size of the patient sample in which microparticle characteristics were determined, further research is needed to establish their significance for the development of adverse COVID-19 progression and outcomes.

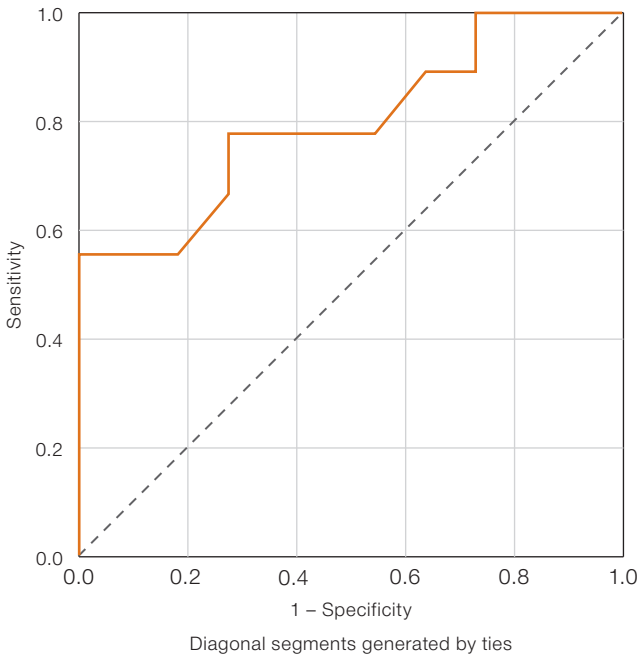

Figure prepared by the authors based on original data

Fig. 3. ROC curves for evaluating the predictive model of platelet-derived microparticles and adverse outcomes in COVID-19

References

1. Galstyan GM. Coagulopathy in COVID-19. *Pulmonology*. 2020;30(5):645–57 (In Russ.). https://doi.org/10.18093/0869-0189-2020-30-5-645-657
2. Bulanov AYu, Roitman EV. New coronavirus infection, hemostatic and heparin dosing problems: it is important to say now. *Thrombosis, Hemostasis and Rheology*. 2020;2:11–8 (In Russ.). https://doi.org/10.25555/THR.2020.2.0913
3. Lobastov KV, Porembskaya OYa, Schastlivtsev IV. Efficiency and safety of antithrombotic therapy in COVID-19. *Ambulatory Surgery*. 2021;18(2):17–30 (In Russ.). https://doi.org/10.21518/1995-1477-2021-18-2
4. Di Minno AD, Ambrosino P, Calcaterra I, Di Minno MND. COVID-19 and venous thromboembolism: a meta-analysis of literature studies. *Seminars in Thrombosis and Hemostasis*. 2020;46(7):763–71. https://doi.org/10.1055/s-0040-1715456
5. Matvienko OYu, Korsakova NE, Lerner AA, Shvedova TN, Papayan LP. The state of the plasma hemostasis in patients with coronavirus infection caused by the SARS-CoV-2 virus. *Thrombosis, Hemostasis and Rheology*. 2020;4:52–6 (In Russ.). https://doi.org/10.25555/THR.2020.4.0945
6. Schulman S, Hu Y, Konstantinides S. Venous thromboembolism in COVID-19. *Thrombosis and Haemostasis*. 2020;120(12):1642–53. https://doi.org/10.1055/s-0040-1718532
7. Kuznetsov SI, Shestakov EA, Zhiburt EB. Coagulopathy in COVID-19 infection. *Thrombosis, Hemostasis and Rheology*. 2020;4:31–4 (In Russ.). https://doi.org/10.25555/THR.2020.4.0942
8. Ranucci M, Ballotta A, Di Dedda U, Baryshnikova E, Dei Poli M, Resta M, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *Journal of Thrombosis and Haemostasis*. 2020;18(7):1747–51. https://doi.org/10.1111/jth.14854
9. Momot AP, Tsarigorodtseva NO, Fedorov DV, Bishevski KM, Vostrikova NV, Klimova EE. Platelet microvesicles and their role in providing hemostatic potential (literature review). *Siberian Scientific Medical Journal*. 2020;40(2):4–14 (In Russ.). https://doi.org/10.15372/SSMJ20200201
10. Zubairov DM, Zubairova LD. *Microvesicles in the blood. Functions and their role in thrombus formation*. Moscow: GEOTAR-Media; 2009 (In Russ.).
11. Sirotkina OV, Ermakov AI, Gaykovaya LB, Kudlay DA, Vavilova TV. Microparticles of blood cells in patients with COVID-19 as a marker of hemostasis activation. *Thrombosis, Hemostasis and Rheology*. 2020;4:35–40 (In Russ.). https://doi.org/10.25555/THR.2020.4.0943
12. Tang K, Wu L, Luo Y, Gong B. Quantitative assessment of SARS-CoV-2 RNAemia and outcome in patients with coronavirus disease. *Journal of Medical Virology*. 2021;93(5):3165–75. https://doi.org/10.1002/jmv.26876
13. Li Y, Shang K, Bian W, He L, Fan Y, Ren T, et al. Prediction of disease progression in patients with COVID-19 by artificial intelligence assisted lesion quantification. *Scientific Reports*. 2020;10(1):22083. https://doi.org/10.1038/s41598-020-79097-1
14. Ozel AS, Altunal LN, Aydin M, Unal M, Cam G, Ozer MC, et al. Clinical characteristics and risk factors associated with severe disease and outcome of patients with COVID-19. *Journal of Infection in Developing Countries*. 2022;16(3):435–44. https://doi.org/10.3855/jidc.15411
15. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clinical Chemistry and Laboratory Medicine*. 2020;58(7):1021–8. https://doi.org/10.1515/cclm-2020-0369
16. Mishchenko TA, Ermakova PA, Ermakova AA, Tseller LP, Rogozhkina YA, Verteletskaya MI, et al. Predictors of severe course of new coronavirus infection (COVID-19): study design. *Therapeutic Archive*. 2022;94(11):1246–51 (In Russ.). https://doi.org/10.26442/00403660.2022.11.201402
17. Bobcakova A, Petriskova J, Vysehradsky R, Kocan I, Kapustova L, Barnova M, et al. Immune profile in patients with COVID-19: lymphocytes exhaustion markers in relationship to clinical outcome. *Frontiers in Cellular and Infection Microbiology*. 2021;11:646688. https://doi.org/10.3389/fcimb.2021.646688
18. Rajpoot A, Mishra M, Banerjee S, Kumar A, Panda PK, Sindhwani G, et al. Predictors of poor outcome in patients with COVID-19 associated respiratory failure: a retrospective observational study. *Journal of the Association of Physicians of India*. 2023;71(4):11–2.
19. Oudkerk M, Büller HR, Kuijpers D, Oudkerk SF, van Beek JR. D-Dimer and COVID-19. *Radiology*. 2020;297(3):343–4. https://doi.org/10.1148/radiol.2020203481
20. Zhang L, Yan X, Fan Q, Liu X, Liu Z, Zhang Z, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *Journal of Thrombosis and Haemostasis*. 2020;18(6):1324–9. https://doi.org/10.1111/jth.14859
21. Momot DA, Mamaev AN, Nikolaeva MG, Momot AP, Kudinov AV, Neimark MI. Advancing plasma D-dimer level determination: an applicability analysis for COVID-19 patients at the hospital stage. *Bulletin of Medical Science*. 2023;2(30):79–86 (In Russ.). https://doi.org/10.31684/25418475-2023-2-79
22. Vorobyeva NA, Vorobyeva AI. Predictive value of D-dimer in COVID-19. *Health Care Standardization Problems*. 2021;5–6:36–42. https://doi.org/10.26347/1607-2502202105-06036-042

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