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Dedicated to our great teachers: A.K. Gus'kova, L.A. Il'in, P.V. Ramzaev, and A.F. Tsyb

MEDICAL AND BIOLOGICAL IMPACTS OF CHERNOBYL NPP ACCIDENT (1986–2026)

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The paper presents the results of a 40-year follow-up of the health status of people exposed due to radiation accident at Chernobyl Nuclear Power Plant (NPP). The issues of radiation hygienic and medical monitoring implementation following Chernobyl NPP disaster in the Russian Federation are considered. Findings of the assessment of deterministic effects, including the lethal ones, in Chernobyl NPP personnel who were affected by high-dose acute external exposure in the first days after the accident are discussed together with the treatment modalities of acute radiation syndrome and its outcomes. Another subject of radiobiological and epidemiological study is long-term cancer and non-cancer effects in nuclear disaster clean-up workers who took part in emergency and recovery operations in 1986–1990 and in residents of the radioactively contaminated territories who were affected by long-term combined (external and internal) radiation exposure at a wide dose range. Health effects for the exposed population offspring, including in utero exposed individuals, are considered separately.

Keywords: Chernobyl NPP; accident; cleanup workers; population, acute radiation syndrome; radiation risk; long-term effects; biomarkers

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П.В. Рамзаеву и А.Ф. Цыбу

МЕДИКО-БИОЛОГИЧЕСКИЕ ПОСЛЕДСТВИЯ АВАРИИ НА ЧЕРНОБЫЛЬСКОЙ АЭС (1986–2026)

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В работе представлены результаты сорокалетних наблюдений за состоянием здоровья людей, подвергшихся радиационному воздействию вследствие аварии на Чернобыльской АЭС. Рассмотрены проблемы организации радиационно-гигиенического и медицинского мониторинга после аварии на территории Российской Федерации. Представлены данные по оценке детерминированных эффектов у аварийных работников АЭС, которые подверглись в первые дни после аварии острому внешнему облучению в высоких дозах, включая летальные; методы лечения острой лучевой болезни и ее исходы. Предметом радиобиологического и эпидемиологического рассмотрения в статье также являются отдаленные канцерогенные и нераковые эффекты у ликвидаторов последствий аварии, участвовавших в аварийно-восстановительных работах в 1986–1990 гг., а также у населения, проживающего на радиоактивно загрязненных территориях, которые подверглись длительному сочетанному (внешнему и внутреннему) радиационному воздействию в широком диапазоне доз. Отдельно рассматриваются последствия аварии для здоровья потомков облученных людей, включая внутриутробно облученных.

Ключевые слова: Чернобыльская АЭС; авария; ликвидаторы; население; острая лучевая болезнь; радиационный риск; отдаленные эффекты; биомаркеры

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INTRODUCTION

The Chernobyl nuclear power plant (NPP) accident, which occurred 40 years ago, was not only the source of the largest uncontrolled release of radioactive substances into the environment in history, but also the most extensive project to mitigate its consequences. More than 500,000 military personnel and workers of various specialties and agencies of the USSR took part in this effort. Following the accident, the state implemented extraordinary measures — first, to address

the immediate consequences of the disaster, and later, to provide medical-social protection and rehabilitation for the irradiated population. After the adoption of Federal Law No. 1244-1 of 1991 “On the Social Protection of Citizens Exposed to Radiation as a Result of the Chernobyl Nuclear Disaster”¹, cleanup workers of 1986–1990 and their children born after the accident, as well as residents of radioactively contaminated territories (RCTs), were guaranteed free medical care and mandatory lifelong special medical monitoring. Under this law, irradiated individuals gained the right to

¹ Law of the Russian Federation No. 1244-1 of May 15, 1991 “On Social Protection of Citizens Exposed to Radiation as a Result of the Catastrophe at the Chernobyl Nuclear Power Plant”.

compensation for damage caused to their health and benefits for the increased risk of developing adverse health effects.

Within the framework of several state and federal targeted programs, scientific research and medico-hygienic measures were carried out to rehabilitate irradiated individuals and RCTs, aimed at improving the health, quality of life, and living conditions of the exposed population. After the accident, experts predicted numerous and highly diverse health consequences for the irradiated population. The forty years that have passed since the accident now allow us to summarize the results of long-term medical observations of irradiated individuals and their descendants, and to draw conclusions about the medico-biological consequences of this tragedy.

The results of medical follow-up of individuals exposed due to the accident and the studies conducted represent significant scientific potential, which enables obtaining fundamentally new knowledge about radiation effects in humans and the mechanisms of their development. The analysis of medico-biological effects was carried out for three human radiation exposure scenarios:

- acute total external exposure at high doses, to which emergency workers at the Chernobyl NPP were subjected on the night of April 26, 1986.
- prolonged, predominantly external exposure at low and intermediate doses, experienced by the accident cleanup workers of 1986–1990.
- chronic combined (external and internal) total exposure at low doses, to which the RCTs population was subjected. In some cases, however, the internal radiation doses to the thyroid gland (TG) were high.

Of considerable interest was also the health status of descendants of individuals exposed as a result of the Chernobyl accident.

MAIN BODY

Medical management and long-term health effects of radiation exposure among nuclear accident emergency responders

Approximately 600 emergency workers (plant personnel and firefighters) received the highest doses. They were exposed to acute high-dose radiation, including lethal doses, in the first days after the accident. The predominant exposure was due to whole-body external gamma radiation at a high dose rate of beta radiation to the skin surface. In contrast, neutron exposure and inhalation intake of radionuclides were negligible.

In the first hours after the accident, medical assistance to the affected individuals was provided by the on-duty staff of the Chernobyl NPP health post and emergency medical services. Twelve hours after the accident, the emergency radiological team from the clinic of the Institute of Biophysics of the USSR Ministry of Health* (hereinafter referred to as the IBPh) commenced operations. The team assessed the radiation situation and conducted medical triage of the irradiated emergency workers to determine the need for and timing of specialized medical care.

After triage of casualties suspected of acute radiation syndrome (ARS), they were transported to the IBPh clinic by special aircraft flights. Within 48 h, 129 irradiated individuals were hospitalized, of whom 84 were diagnosed with moderate, severe, and extremely severe ARS. At the clinic, radiation dose verification was performed using cytogenetic methods [1]. The activity of incorporated radionuclides was measured using human radiation counters² [2]. Radiation doses received by ARS patients from whole-body external gamma radiation and in the bone marrow ranged 0.8–16 Gy. Skin doses from beta radiation, estimated in eight ARS patients, were 10–30 times higher than whole-body doses from external radiation and reached 400–500 Gy³ [3].

Of the 134 patients with verified ARS, 28 individuals died within the first four months. The primary cause of death was acute bone marrow syndrome, induced by exposure to high doses of gamma radiation. The immediate causes of death included infectious complications, intoxication, and progressive multiple organ failure [3]. Additionally, two emergency workers died shortly after the accident from injuries sustained during the incident [4].

Allogeneic bone marrow transplantation was performed in thirteen patients. Six additional patients received transplants of human fetal liver cells. All of these patients died, except for one individual, in whom, as later discovered, the activity of the native bone marrow recovered, leading to transplant rejection. In three cases, the fatal outcome may have been associated with unsuccessful bone marrow transplantation. Based on the treatment results for patients with ARS, it was concluded that bone marrow transplantation is indicated only for patients with irreversible suppression of hematopoiesis caused by acute total relatively uniform gamma radiation exposure at doses of 8–10 Gy [4, 5].

All patients with severe and extremely severe bone marrow syndrome had radiation-induced skin lesions. These lesions exacerbated suppression of hematopoiesis, especially in cases of damage to 50% of the body surface, and were complicated by infections. It is assumed that skin injuries played a major role in at

* Currently, the clinic of the State Scientific Center of the Russian Federation — Burnasyan Federal Medical Biophysical Center of Federal Biological Agency.

² Ilyin LA, ed. Radiation medicine. Manual for medical researchers, public health managers. Moscow: IzdAT; 2001 (In Russ.).

³ Barabanova AV, Baranov AE, Bushmanov AY, Guskova AK. Radiation damage to humans: selected clinical lectures, methodological guide. Moscow: Slovo; 2007 (In Russ.).

least nineteen fatal outcomes and significantly aggravated the course of ARS [2, 6]. Patients whose skin did not heal within 50–60 days underwent skin grafting. Gastrointestinal syndrome was observed in fifteen patients, and radiation pneumonitis in eight.

Subsequent long-term follow-up of individuals who survived ARS showed nearly complete restoration of hematopoiesis within several months after radiation exposure, whereas recovery of the immune system took at least six months. Full normalization of T-cell immunity required several years.

The main long-term medical consequences of ARS were radiation-induced skin lesions and cataracts. Skin injuries depended on the radiation dose and the severity of ARS, ranging from mild degenerative changes to the development of severe scars and ulcers. For example, between 1990 and 1996, 15 individuals with extensive skin lesions underwent surgical treatment. Radiation cataracts developed in many patients with moderate and severe ARS during the first few years after the accident.

Most men who survived ARS experienced sexual dysfunction until 1996; however, 14 healthy children were born to some of them within the first five years after the accident.

In the first decade after the accident, individuals who had survived ARS showed a high prevalence of nervous system disorders, cardiovascular diseases, and gastrointestinal diseases. However, the incidence of these conditions did not correlate with the ARS severity, suggesting that these diseases were more likely of non-radiation origin.

Among the causes of death in the long-term follow-up period, non-neoplastic systemic diseases predominated — specifically, disorders of the cardiovascular system, respiratory system, and digestive system. Neoplasms (cancer) were less common [7]. Given the stochastic nature of the pathology leading to fatal outcomes in individuals who survived ARS, the relationship between long-term causes of death and radiation exposure is not evident. The fatal cases appear to have been caused by non-radiation factors.

Medical and biological consequences of radiation exposure in accident cleanup workers

As noted earlier, more than 500 thousand various specialists participated in the emergency response to the accident and subsequent recovery operations. June 1986 marked the beginning of the formation of the All-Union Distributed Registry of Individuals Exposed to Radiation. This registry included Chernobyl accident cleanup workers and later became the foundation of the National Radiation and Epidemiological Registry [8, 9].

Radiation monitoring and radiation doses received by cleanup workers. The release of radioactive materials

from the damaged reactor began on April 26, 1986, and, according to some estimates, continued for 10–15 days. Short-lived radionuclides — such radioisotopes as ^{131}I , $^{103,106}\text{Ru}$, ^{144}Ce , ^{140}Ba , etc. — were the main contributors to radiation doses at the early stage of the accident. Later, cesium radioisotopes (^{137}Cs , ^{134}Cs) played an increasingly significant role in dose formation, while strontium isotopes (^{90}Sr) contributed much less. Radioisotopes of transuranic elements ($^{238,239,240,241}\text{Pu}$, ^{241}Am), although present in the radioactive releases, made a negligible contribution to the total radiation dose [10]. The primary exposure pathway for cleanup workers was external gamma radiation from radioactive materials deposited on the ground and building surfaces.

Radiation surveillance and exposure doses among Chernobyl accident cleanup workers. The release of radioactive materials from the damaged reactor began on April 26, 1986, and, according to some estimates, continued for 10–15 days. Short-lived radionuclides — such radioisotopes as ^{131}I , $^{103,106}\text{Ru}$, ^{144}Ce , ^{140}Ba , and others — were the main contributors to radiation doses at the early stage of the accident. Later, cesium radioisotopes (^{137}Cs , ^{134}Cs) played an increasingly significant role in dose formation, while strontium isotopes (^{90}Sr) contributed much less. Radioisotopes of transuranic elements ($^{238,239,240,241}\text{Pu}$, ^{241}Am), although present in the radioactive releases, made a negligible contribution to the total radiation dose [10]. The primary exposure pathway for cleanup workers was external gamma radiation from radioactive materials deposited on the ground and building surfaces.

Dosimetric monitoring of cleanup workers was organized on a large scale approximately one month after the accident [11]. Even among cleanup workers seconded from enterprises of the USSR Ministry of Medium Machine Building, less than half of the personnel were provided with individual dosimeters [12]. By decision of the USSR Government Commission dated May 28, 1986, individual dosimetric control (IDC) was assigned to three agencies: the USSR Ministry of Defense, the USSR Ministry of Medium Machine Building, and the USSR Ministry of Energy. The USSR Ministry of Internal Affairs and the USSR Committee for State Security also carried out dosimetric monitoring independently until September 1987. Since 1986, personnel from more than 600 organizations across 49 ministries and agencies of the USSR were subject to IDC. The largest number of cleanup workers were assigned to units of the USSR Ministry of Energy [12].

The dosimetric data collected in the National Radiation and Epidemiological Registry (NRER) can be divided into three types:

- values from an individual dosimeter worn by a specific cleanup worker;
- group dose values based on readings from an individual dosimeter worn by one member of the group;

- estimates of route specific doses, derived from the average exposure rate in the work area and the duration of the group's presence there.

Currently, 142.9 thousand participants in the accident cleanup have information on external radiation doses received in the accident zone, which accounts for 71.4% of the total number of cleanup workers registered in the NRER. For comparison: in the Japanese registry of survivors of the atomic bombings of Hiroshima and Nagasaki, out of 120,300 registered individuals, 86,600 people (72.0%) have data on reconstructed individual doses. An analysis of the quality of dosimetric data for cleanup workers entered into the NRER showed no obvious contradictions between the parameters of the radiation environment in radioactive contamination zones, the nature of the work performed, and the doses recorded in the registry. Furthermore, a statistical analysis of dosimetric data for cleanup workers registered in the NRER revealed a small proportion of individuals whose doses lack an acceptable degree of reliability [12].

The period of radiation exposure for cleanup workers was limited to several months [9]. The accumulated average external radiation dose for all cleanup workers, regardless of the year they entered the accident zone, was 106 mGy. Cleanup workers who entered the accident zone in 1986 received, on average, one-and-a-half times higher radiation doses (more than 150 mGy). The distribution of the average dose rate among cleanup workers who received doses exceeding 150 mGy followed a log-normal distribution, with a mean value of approximately 3 mGy/day. Due to the established regulatory limit for radiation exposure during the response to radiation accidents, the total radiation doses for 97.5% of cleanup workers did not exceed 250 mGy.

Health monitoring and vital status. The NRER is a state information system containing personal data of individuals exposed to radiation as a result of the Chernobyl nuclear power plant accident, other radiation accidents and incidents, and nuclear tests. It was created to ensure lifelong monitoring of health changes in such individuals. In accordance with Russian legislation, the NRER registers citizens of the Russian Federation who belong to one of 24 accounting categories. Twelve of these categories pertain to individuals exposed to radiation due to the Chernobyl accident, as well as their descendants. The NRER system operator is the Ministry of Health of the Russian Federation. The lead organization within the NRER system is the A. Tsyb Medical Radiological Research Centre — branch of the National Medical Research Radiological Centre.

The NRER is a geographically distributed information system that includes: a unified federal database, regional segments (in the constituent entities of the Russian Federation), and four departmental sub-registries (under the Ministry of Defense of Russia, the Ministry of Internal Affairs of Russia, the Ministry of Emergency Situations

of Russia, and the Federal Medical-Biological Agency of Russia). The regional segments of the registry are maintained by the authorized executive bodies of all constituent entities of the Russian Federation [11].

Currently, 834,200 individuals are registered in the NRER, of which more than 763 thousand belong to the "Chernobyl cohort", including:

- more than 200,000 cleanup workers involved in the aftermath of the accident; more than 370,000 residents of the Bryansk, Kaluga, Oryol, and Tula regions who live in the resettlement zone and the zone with the right to resettlement;
- more than 50 thousand descendants of the cleanup workers.

Collection of individual medical and dosimetric information is carried out at the regional and municipal levels during mandatory special medical surveillance (specialized preventive health check-up) and in the process of seeking medical care. The average annual coverage rate of cleanup workers by medical examinations, both over the entire observation period as a whole and over the past 10 years, is approximately 80%. In the first 15 years after leaving the radiation-affected zone, the rates of primary and overall morbidity among cleanup workers for many disease classes significantly exceeded the corresponding rates for reference population groups in the Russian Federation matched by age and sex characteristics. In subsequent years, this excess steadily declined. Primarily, the excess morbidity is explained by the implementation of specialized диспансеризация among cleanup workers. The only disease class demonstrating a steady increase in primary morbidity throughout the entire observation period is the Neoplasms class.

The overall mortality rate among cleanup workers over the entire observation period is statistically significantly lower (by an average of 15%) than the mortality rate in the reference population group of the Russian Federation matched by age and sex characteristics. Their longer life expectancy is explained, on the one hand, by the so-called "healthy worker effect", and on the other hand, by a higher level of socio-medical care provided to cleanup workers. The mortality rate from malignant neoplasms (MN) over the entire observation period was statistically significantly higher than the control rate in the reference group of the male population of the country — by 6%. According to NRER data as of early 2026, approximately 52.5% of the total number of cleanup workers registered in the registry have died, of whom:

- 42.7% died from cardiovascular system diseases;
- 18.5% died from malignant neoplasms;
- 15.8% died from injuries and poisonings.

Radiobiological effects. Preclinical effect studies received particular attention, as they were considered biological markers of individual radiation dose and predisposition to radiation-induced pathology. Cytogenetic and

molecular genetic changes in peripheral blood lymphocytes (PBL) of cleanup workers proved to be sufficiently reliable biomarkers of radiation exposure. In the early period after the accident, a high level of unstable-type chromosomal aberrations (dicentrics and centric rings) was observed [13, 14], which decreased over time but remained statistically significantly elevated compared to non-exposed individuals for more than twenty years [15]. In the long-term follow-up period, the level of stable translocations was recorded, detected by fluorescence *in situ* hybridization (FISH) [16]. However, good comparability of cytogenetic data with physical dosimetry was noted only for a subset of the examined cleanup workers. At the same time, a high individual variability in the frequency of stable chromosomal aberrations induced by low radiation doses was observed [17]. In subsequent years, the level of translocations in PBL decreased to a certain plateau, but its value clearly correlated with the initial radiation dose estimate, which confirms the significance of stable markers for retrospective dosimetry [18].

The results of somatic mutagenesis assessment based on the analysis of mutations in the T-cell receptor gene of blood lymphocytes (TCR mutations) indicate an increase not only in the early period after radiation exposure, but also after a long time has elapsed. For example, in a subset of cleanup workers exposed to radiation doses up to 2 Gy, an increased frequency of TC-mutant lymphocytes was detected in the long-term period after the accident. Moreover, this indicator remained virtually unchanged over time following radiation exposure [19, 20].

For many years, dose-dependent changes in adaptive immunity, predominantly T-cell-mediated, persisted among cleanup workers. In the early period after radiation exposure, an increase in the number of T-lymphocytes and T-helper cells was observed compared to non-exposed individuals. The most active increase in the T-helper cell population was recorded in cleanup workers exposed to low radiation doses [21]. However, 3 months after radiation exposure, a decrease in the levels of T-lymphocytes and T-helper cells was observed, which also correlated with the radiation dose. In later follow-up periods, the reduced frequency of CD3⁺ cells and CD3⁺CD4⁺ cells exhibited a persistent pattern [22, 23], manifesting as:

- an imbalance in the CD4⁺/CD8⁺ ratio⁴;
- an increased level of regulatory T-cells in the blood [24];
- elevated levels of pro-inflammatory cytokines IL-1 β , IL-6, IFN- γ , and TNF- α in blood serum [23].

Medical consequences. No ARS cases were registered among cleanup workers. The main concern was long-term effects, which were not specific to radiation exposure (multifactorial pathology) and had a stochastic nature. The probability of their development is

determined by the radiation dose. The most reliable data on the consequences of radiation exposure among cleanup workers were obtained from a cohort of 66,000 male cleanup workers who entered the accident zone in 1986–1987. This cohort is supported by dosimetric data and health status information for the entire observation period. The average age of the cleanup workers at the start of the observation period was 34 years [25].

In the structure of leukemia incidence over the period 1986–2007 (198 cases registered) [26], chronic forms predominated, with equal proportions of myeloid and lymphoid types. A statistically significant excess risk of leukemia incidence, excluding chronic lymphocytic leukemia (CLL), was observed only during the first ten years after the accident — both when compared to national rates and when using internal control. The radiation risk for CLL was statistically insignificant. The excess of the incidence rate in the cohort over the corresponding rate for the Russian male population during the period 1986–1997 amounted to 89% (age-standardized ratio 1.89 (95% CI [1.42; 2.45]) [27]. Subsequently, in 1998–2018, this indicator was 23% lower than the Russian rate (age-standardized ratio 0.77 (95% CI [0.64; 0.93])) [27].

When using the linear no-threshold (LNT) model of radiation risk, the excess relative risk coefficient per 1 Gy (ERR/Gy) for the first ten years after the accident was 4.41 (90% CI [0.24; 14.23]), and subsequently became statistically insignificant [27]. According to the LNT model, approximately 32% of leukemias (excluding CLL) in the first ten years after radiation exposure can be attributed to radiation effects.

Since follow-up monitoring of cleanup workers in 1998–2025 confirmed the absence of radiation-related leukemia risk, it can be assumed that radiation-induced leukemias in cleanup workers had already fully manifested by 1998. The excess number of such cases amounted to 8, which is the average value between the lifetime predictions of the International Commission on Radiological Protection (ICRP) and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). The lifetime projection of radiation-induced leukemia cases among cleanup workers after 2020 is estimated at 1 case according to the ICRP model and 18 cases according to the UNSCEAR model.

Analysis of the dose-response relationship showed agreement between estimates of relative radiation risk (RR) from the LNT model and non-parametric RR estimates relative to the control group of cleanup workers with radiation doses below 0.05 Gy, taking into account their 90% confidence intervals [27]. Statistically significant RR estimates were observed only at doses exceeding 0.15 Gy. Thus, for radiation doses below 0.15 Gy, the LNT model of radiation leukemia risk in cleanup workers was conservative and led to overestimated radiation risk assessments [27].

⁴ Pashchenkova YuG. Monitoring the immune status and its prognostic significance for the early diagnosis of malignant neoplasms in liquidators of the consequences of the Chernobyl nuclear power plant accident. Abstract of the dissertation ... candidate of medical sciences; 2013.

The incidence of solid malignant neoplasms (MNs) during the observation period 1992–2019 was 11% higher than the rate for the Russian male population (95% CI [9; 13]). At the same time, mortality from MNs did not differ from the national average for Russia. Within the framework of the LNT model, the excess relative risk per 1 Gy (ERR/Gy) for the incidence of solid MNs was 0.62 (95% CI [0.29; 0.98]), and for mortality, ERR/Gy was 0.74 (95% CI [0.32; 1.22]). Radiation risks of incidence and mortality from solid MNs were mainly determined by neoplasms of the esophagus, stomach, and colon (ERR/Gy): 0.89 (95% CI [0.20; 1.74]) for incidence, and ERR/Gy at the level of 0.77 (95% CI [−0.05; 1.81]) for mortality. ERR/Gy for the incidence of MNs of the trachea, bronchi, and lung was 0.85 (95% CI [0.21; 1.62]), and for mortality, 0.85 (95% CI [0.13; 1.72]). This can be partially attributed to the highest prevalence of tumors in these locations, which account for more than half of all cases of solid MNs.

A quantitative comparison of projected estimates of lifetime radiation risk of MNs, calculated using radiation risk models from the ICRP, the UNSCEAR, and the NRER, in the cohort of cleanup workers showed that when using own estimates after 2020, 1297 excess cases of MNs are expected in the cohort of Russian cleanup workers. This is 2.4 times higher than the values obtained from calculations using international models.

As the follow-up period increases, the trend of a slight increase in radiation risks for solid malignant neoplasms (MNs) persists after 2019. The latest ERR/Gy estimates, made as of early 2026, are 0.73 (95% CI [0.43; 1.07]) and 0.76 (95% CI [0.37; 1.20]) for incidence and mortality, respectively. Non-parametric RR estimates in dose intervals, similarly to those for leukemias, were statistically significant only for radiation doses of 0.15 Gy and higher [27]. This indicates the conservativeness of radiation risk estimates for solid MNs in cleanup workers in the low-dose radiation exposure range when using the LNT model.

Follow-up monitoring of the cleanup worker cohort showed an increase in the incidence of blood disorders (ICD-10: D50–D89). In this case, the LNT model adequately describes the risk magnitude at various levels of radiation exposure, with ERR/Gy equal to 0.59 (95% CI [0.23; 0.99]) [8]. Of note are the high radiation risks for the development of aplastic anemias (ICD-10: D60–D61), where ERR/Gy was 5.56 (95% CI [0.53; 20.67]). For blood and hematopoietic organ disorders not classified as anemias or hemorrhages (ICD-10: D70–D77), ERR/Gy was 2.25 (95% CI [0.97; 3.95]), and for immunodeficiency states (ICD-10: D80–D89), ERR/Gy was 2.01 (95% CI [0.53; 4.10]). In contrast to leukemias, for which the radiation risk manifested within the first ten years after exposure [6], the excess risk of aplastic anemias has persisted for over 30 years.

The ERR/Gy incidence coefficient for cardiovascular system diseases (ICD-10: I00–I99) is statistically

significant and amounts to 0.57 (95% CI [0.46; 0.68]), as is the mortality coefficient — 0.42 (95% CI [0.19; 0.60]). It should be noted that in the Japanese cohort of atomic bomb survivors, the radiation risk of mortality from cardiovascular system diseases for the male population was estimated at a 6-fold lower ERR/Gy coefficient, amounting to 0.07 (95% CI [−0.001; 0.16]) [28].

Non-parametric RR estimates based on mortality data confirm the validity of the LNT model, which contradicts the ICRP's claims of a threshold relationship between the incidence of cardiovascular system diseases and radiation dose level, with a threshold value of 0.5 Gy.

The results of recent studies on cataract incidence (ICD-10: H25–H26) indicate a significant dependence of the prevalence rate of this nosology on the dose of external gamma radiation [11]. The obtained estimates of dose thresholds do not support the need to reduce the maximum permissible dose to the eye recommended by the International Commission on Radiological Protection (ICRP) [29].

The results of non-parametric RR model assessments indicate that the radiation effect, manifested as an increase in the incidence of endocrine system diseases, nutritional disorders, and metabolic abnormalities (ICD-10: E00–E90), as well as nervous system diseases (ICD-10: G00–G99), may be threshold-based [30]. The greatest contribution to excess morbidity from endocrine system diseases comes from iodine deficiency (E01), goiter (E04), thyroiditis (E06), and type 2 diabetes mellitus (E11). For nervous system diseases, the main contributors are polyneuropathies and other disorders of the peripheral nervous system (G60–G64), as well as other nervous system disorders (G90–G99).

A detailed analysis of the risk of respiratory system diseases and digestive system diseases allowed estimating the parameters of the LNT model of radiation risks [31, 32]. Non-parametric RR estimates confirmed the validity of the LNT models. The average ERR/Gy coefficient for morbidity from respiratory system diseases across the entire cohort of cleanup workers was 0.21 (95% CI [0.11; 0.32]), and for digestive system diseases — 0.33 (95% CI [0.22; 0.44]). Increased morbidity from respiratory system diseases is driven by conditions such as influenza and pneumonias (J09–J18), other upper respiratory tract diseases (J30–J39), as well as chronic lower respiratory tract diseases, including chronic obstructive pulmonary disease (COPD, J44) and asthma (J45–J46). The radiation risk of morbidity from digestive system diseases is primarily attributable to gastric and duodenal ulcers, gastritis and duodenitis, chronic hepatitis not classified elsewhere, as well as gallbladder and biliary tract diseases.

Statistically significant radiation risks have also been obtained for mental disorders (F00–F99), musculoskeletal system diseases (M00–M99), and genitourinary system diseases (N00–N99), confirming the need for a more detailed analysis of the incidence of these nosologies as

a function of radiation dose [33]. It should be noted that for infectious and parasitic diseases (A00–B99), ear diseases (H60–H95), benign neoplasms (D10–D36), as well as skin and subcutaneous tissue diseases (L00–L99), no radiation risk was detected in cleanup workers despite their high prevalence [16].

An assessment of the dynamics of radiation risks for morbidity among cleanup workers from major non-cancer diseases shows that the values of these risks over the entire follow-up period are determined by disease cases that arose in the first 10–15 years after the accident. It is important to note that the radiobiological mechanisms underlying the increase in morbidity and mortality from the aforementioned non-cancer diseases are insufficiently studied. The most likely pathogenetic mechanism of multi-organ pathology in cleanup workers is chronic non-specific inflammation induced by radiation exposure, which is mediated by a pro-inflammatory immune status [34].

Medical consequences for the RCT population of the Russian Federation

Emergency releases of radioactive compounds have caused contamination of territories in the Russian Federation, particularly in the Bryansk, Kaluga, Oryol, and Tula regions.

Radiation-hygienic monitoring and population radiation doses. Operational dosimetric monitoring of the radiation situation and its dynamics was organized in the very first days after the accident. The purpose of radiation-hygienic monitoring — both at the early stage of a radiation accident and at its current stage — is to obtain a set of initial data required for calculating radiation doses received by residents of settlements located in RCTs. In the early period, radiation doses were primarily used to develop protective measures and assess their effectiveness. In subsequent periods, monitoring makes it possible to analyse the dynamics of the radiation situation and evaluate the medical-biological consequences of the accident. Depending on changes in the situation (due to the physical decay and migration of radio-nuclides that are significant at a given time) and the monitoring objective, the set of monitored parameters is subject to change.

External exposure of the population is caused by the decay of gamma-emitting radio-nuclides present in the environment. Immediately after the accident, the elevated gamma background was determined by short-lived radio-nuclides (^{131}I , $^{103,106}\text{Ru}$, ^{144}Ce , ^{140}Ba , etc.), and later — by cesium radioisotopes (^{134}Cs и ^{137}Cs). In the first weeks after the accident, iodine isotopes were the main dose-forming radio-nuclides for internal exposure of the

population. The primary route of their intake into the bodies of RCTs residents was through consumption of cow's milk and fresh greens. From the third decade of May 1986, ^{134}Cs и ^{137}Cs became the main dose-forming nuclides, entering the body mainly via milk and meat, as well as through plant-based and natural food products of local origin. The contribution of strontium isotopes ($^{89,90}\text{Sr}$) to the internal radiation dose of the Russian population is insignificant (1–5%).

In settlements classified as radioactive contamination zones, systematic monitoring of the radiation situation is organised. The settlement survey programme is developed taking into account:

- their classification as radioactive contamination zones;
- the priority of settlements with high levels of ^{137}Cs soil contamination.

The most extensive monitoring is carried out in the heavily contaminated Bryansk region. Residents of some settlements undergo individual dosimetric monitoring using TLD dosimeters. As part of radiation-hygienic food monitoring, radio-nuclide content (^{137}Cs and ^{90}Sr) is analysed in milk and potatoes produced in residents' private households, mushrooms. In settlements, systematic measurements of the exposure rate of gamma radiation are conducted⁵ [35].

To determine population protection measures, methodologies have been developed for assessing radiation doses both under conditions of no active radiation protection measures and for actual radiation doses — on average for residents of a settlement, for a critical group of residents, and for accumulated radiation dose⁶. To classify a settlement into a specific zone, the average annual effective dose (AAED₉₀) among residents is estimated for living and conducting economic activities without active radiation protection measures. When assessing the average accumulated effective dose (AAED), results from actual radiation dose assessments for the population are used. The most reliable information on actual population exposure levels is obtained from radiation monitoring data collected in contaminated territories. Based on these results, the average annual effective dose for residents of a settlement (AAED-S) is estimated. The average annual effective dose for the critical group of settlement residents is defined as the mean dose received by the top 10% of residents who have the highest individual radiation doses compared to the rest of the population.

By 2020, the average effective dose for the critical population group in 386 settlements of the Bryansk, Tula, Oryol, and Kaluga regions amounted to approximately 40 mSv [36]. The highest thyroid doses were received by the population evacuated from the 30 km zone. The average dose was about 500 mGy, while thyroid radiation doses in children under three years of age

⁵ On the state of sanitary and epidemiological well-being of the population in the Bryansk Region in 2024. State Report. Bryansk: Department of Rosпотребнадзор for the Bryansk region; 2024 (In Russ.).

⁶ Assessment of radiation doses to the population of the Russian Federation due to the Chernobyl accident: Collection of methodological documents. 3rd edition. St. Petersburg; 2011.

reached 50 Gy [37]. Average thyroid radiation doses from ^{131}I among residents of settlements located in areas with a ^{137}Cs contamination density exceeding 37 kBq/m², in children under one year of age, were as follows:

- Bryansk region — up to 1.3 Gy;
- Kaluga region — 0.16 Gy;
- Oryol region — 0.23 Gy;
- Tula region — 0.19 Gy.

Maximum individual doses in children under three years of age in the Bryansk region reached 10 Gy [38].

Medical and biological consequences of radiation exposure in the population. Analysis of medical consequences prior to the establishment of the irradiated population registry was carried out using the geographical method. A distinctive feature of this method is the assessment of radiation effects on the population of a specific territory (for example, a district or region) using nationwide (or other) statistical data on population size, its dynamics, and age-and-sex structure. Such studies use average group doses estimated for individual territories (districts) and age groups.

After the NRER establishment in the Russian Federation, it became possible to conduct analytical studies (cohort studies, case-control studies) that provide reliable information on the medical consequences of radiation exposure. These studies are based on long-term health monitoring of the population throughout their lives after the accident, using individual radiation doses of cohort members. According to current standards, all individuals registered in the NRER undergo annual medical examinations, which ensures high completeness and quality of the data.

The results of cytogenetic studies conducted among evacuees and RCTs residents — carried out to assess radiation doses in the first years after the accident — indicated an increased level of aberrant lymphocytes in blood and unstable-type chromosomal aberrations compared to non-irradiated individuals [39]. Later, an increase in the frequency of stable-type chromosomal aberrations (predominantly translocations and inversions) in peripheral blood lymphocytes (PBLs), detected by the FISH method, was established [40]. Stable-type chromosomal aberrations persisted for over 20 years and indicated the possibility of cytogenetic dose assessment nearly 30 years after the accident using the FISH method [16, 18, 41].

Regarding medical effects, no ARS cases or tissue radiation reactions were observed in the population, as well as in cleanup workers. Concerns were raised about multifactorial diseases, the likelihood of developing which could increase in the long term after radiation exposure. However, long-term analysis of the incidence of leukaemias, solid malignant neoplasms (MNs) of various localisations, non-cancer diseases (diseases of the circulatory system, respiratory system, thyroid gland, etc.) in the irradiated population did not reveal any influence

of radiation exposure on their incidence rates — with the exception of thyroid cancer (TC).

According to the NRER, the radiation risk of leukaemia for the RCTs population is not statistically significant, although the average value of excess radiation risk still exceeded the zero level and amounted to 7%.

The radiation risk of developing solid MNs, including breast cancer risk in women, for the population of radioactively contaminated territories is also not statistically significant. This is explained by the low doses of total population exposure. If conservative risk projections based on ICRP risk models are followed, the excess radiation risk of morbidity from solid MNs among the population should be expected at a level 1% above spontaneous morbidity rates. With such a small expected risk, a longer period of health monitoring of the population living in contaminated territories will be required to detect it.

A distinctive feature of population exposure following the Chernobyl accident was the high-dose ^{131}I impact on the TG in individuals who were children or adolescents at the time of the accident. The increased TC incidence in this group remains the only proven effect of radiation exposure among populations living in contaminated territories [11, 42, 43]. For example, in a large-scale case-control study conducted among individuals residing in the most contaminated areas of Belarus and the Russian Federation, the odds ratio for TC development at a dose of 1 Gy was 5.2 (95% CI [2.2; 8.2]) [42]. The results are generally consistent with estimates from studies in cohorts of residents of:

- Ukraine: relative risk, RR/Gy — 2.9 (95% CI [1.4; 7.3]);
- Belarus: RR/Gy — 3.2 (95% CI [1.8; 6.5]);
- Russian Federation: RR/Gy — 5.6 (95% CI [3.7; 8.0]) [43–45].

The excess risk was detected 4–5 years after exposure and, despite its decline over time, remains statistically significant for decades after the accident. This is due not only to the carcinogenic effect of ^{131}I , but also to TC screening effect. Analysis of the Russian cohort showed that in the period 1991–1995, the screening coefficient for children and adolescents was at its maximum and amounted to 11.9. After 1996, however, the coefficient decreased by half [43].

It should be noted that the value of the excess relative risk per gray (ERR/Gy) for TC in individuals exposed to radiation during childhood and adolescence shows high variability across different studies. This necessitates continued research into the dependence of TC risk — primarily on iodine deficiency, the influence of non-radiation factors, and uncertainties in thyroid radiation dose estimates. At the same time, convincing evidence has been obtained that individuals who took stable iodine supplements in schools and summer camps after the Chernobyl accident had a significantly lower TC risk compared to those who did not take iodine supplements. Research findings demonstrate the critical

importance of prophylactic intake of stable iodine during nuclear power plant accidents — especially in regions with iodine deficiency [42].

Estimates of the proportion of radiation-induced TC cases also show high variability. The UNSCEAR estimates the proportion of excess radiation-associated cancer cases at 60% for children and 25% for adolescents, respectively⁷. In the Russian cohort of children residing in the most contaminated areas, it was shown that out of 423 cancer cases identified in 1991–2019, 21% (i.e., 87 cases) can be attributed to radiation exposure. At the same time, among the group of children under 4 years of age, 58% (i.e., 60 cases) are radiation-induced. The difference in the proportion of radiation-induced cases is mainly determined by significantly higher thyroid radiation doses in children under 4 years compared to older age groups [43].

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Molecular mechanisms of TC in irradiated individuals. Molecular genetic studies are aimed at investigating radiation-specific genetic changes in thyroid cancer (TC) cells in individuals exposed to radiation during childhood. This allows establishing the radiation etiology of cancer in each specific case. Particular attention has been paid to gene rearrangements; deletions; point mutations; gene copy number increases; aberrant methylation in genes responsible for cell proliferation, differentiation and apoptosis. The following candidate genes were analysed: *BRAF*, *HRAS*, *KRAS*, *NRAS*, *PTEN*, *PIK3CA*, *AKT1*, *CTNNB1*, *TP53*, *IDH1*, *EGFR*, *HCTC*, *RET*, *NTRK1* and *TERT* (TERTp). The dysfunctional activity of these genes plays a significant role in the oncogenesis of follicular and papillary TC. As potential oncological markers, genes encoding receptors of microenvironment cells were also considered, in particular: *CXCL10*, *AGTR1*, *CTGF*, *FAM3B*, *IL11*, *IL17C*, *PTH2R* and *SPAG11A* [46].

It has been established that tumour growth and progression of TC are closely linked to somatic point mutations in the *BRAF*, *RAS* and *RET* genes. Activation of these genes through mutations or rearrangements is the main initiator of TC development via a signalling pathway involving mitogen-activated protein kinase (MAPK)

and phosphoinositide-3-kinase (PI3K). This enhances nuclear translocation of the *ERK* gene and modifies the transcription of target genes [47, 48].

It is assumed that molecular rearrangements caused by radiation exposure and initiating malignant transformation of thyroid follicular cells may be unique and specific to radiation exposure. In particular, in the first ten years after the Chernobyl accident, variants of papillary thyroid cancer (PTC) with *RET/PTC3* rearrangements were quite common. In later periods, however, *RET/PTC1* rearrangements became predominant [49, 50].

The *RET* gene encodes a membrane protein of the tyrosine kinase receptor family. Under normal conditions, it is either not expressed or expressed at a very low level in thyroid follicular cells and is classified as a proto-oncogene. *RET* activation can occur through chromosomal inversion or translocation, which lead to fusion of the coding region of the *RET* intracellular domain with the 5'-end of heterologous genes. The resulting chimeric *RET/PTC* sequence encodes oncoproteins that trigger the development of papillary thyroid carcinoma. To date, more than 13 *RET/PTC* variants have been registered. These variants differ in terms of the 5'-partner gene involved in the rearrangement. However, the most common types in thyroid cancer cells are: *RET/PTC1* (fusion with the *CCDC6* gene), *RET/PTC3* (fusion with the *NCOA4* gene) [51]. Despite early research findings indicating that *RET/PTC* rearrangements are specific to radiation-induced TC, current evidence suggests that they are also quite common in sporadic TC [49, 52, 53].

It is likely that the more frequent detection of *RET/PTC3* and *RET/PTC1* rearrangements in TC cells is due to the high sensitivity of these regions to genetic damage — not only as a result of ionising radiation exposure, but also due to other genotoxic factors. *RET/PTC* rearrangements are now recognised as the predominant driver mutations not only in radiation-induced cancer, but also in sporadic PTC in children [54].

The V600E point mutation in the *B-RAF* oncogene (a substitution of thymine with adenine at nucleotide 1799) is one of the most frequent mutations in cells of sporadic PTC in adults, and is rarely found in follicular cancer cells [55]. This mutation promotes tumour initiation and metastasis [56]. However, in PTC cells from individuals exposed to radiation after the Chernobyl accident, the *B-RAF* V600E mutation was significantly less common than *RET/PTC* rearrangements [57].

Thus, the question of the existence of specific radiation-induced molecular markers of TC remains open.

Health status of descendants

According to the UNSCEAR and the ICRP, there is currently no convincing evidence in humans that parental exposure to ionising radiation leads to any health changes in their descendants. However, in children

⁷ Evaluation of data on thyroid cancer in regions affected by the Chernobyl accident. New York: United Nations; 2018.

exposed *in utero* as a result of the atomic bombings of Hiroshima and Nagasaki, a reduction in cognitive abilities has been observed. This reduction is dependent on both the radiation dose received and the gestational age at the time of exposure. These findings have generated significant interest in similar studies following the Chernobyl accident.

Transgenerational effects. Special attention is focused on the possibility of identifying transgenerational effects in first-generation descendants who were not themselves exposed to radiation, but were born to accident liquidators after their radiation exposure or to RCTs residents. Such effects, which have been documented only in animal experiments, are attributed to pre-conceptional (before conception) irradiation of parental gonads. When assessing the health status of descendants, a wide range of health parameters were evaluated:

- anthropometric indicators of newborns;
- incidence of various pathologies, including MNs and leukaemias;
- immune status;
- frequency and pattern of congenital malformations;
- perinatal, infant and child mortality;
- and other parameters.

As a result of radiological epidemiological studies in a cohort of children of liquidators registered in the NRER; includes 11 698 liquidator fathers and 15,450 of their children born after leaving the accident zone, no statistically significant association was found between the MNs incidence and congenital malformations in liquidators' children and the level of external radiation dose received by their fathers [58]. In children born in resettlement zone territories and in zones with the right to resettlement, no statistically significant dependence of the incidence of congenital malformations on the radiation dose received by their parents has been identified to date. A conservative estimate, performed in accordance with the 2007 ICRP recommendations, showed that the possible proportion of radiation-induced hereditary diseases in descendants born to parents who lived in RCTs before 2000 may reach 0.4% of the national average indicators for Russia⁸.

When evaluating the results of clinical and epidemiological observations of descendants, it is necessary to note their inconsistency [58–60]. It is noteworthy that in cases where effects were detected in descendants, no evidence was provided to link them to the genetic radiation dose received by the parents. The authors also fail to take into account that the descendants themselves lived in RCTs and were exposed to both in utero and postnatal radiation. Other important challenges in transgenerational effect studies include insufficient statistical power of the studies and low levels of parental gonadal radiation doses [61].

In several studies, authors explain the observed health changes in descendants by radiation-induced genomic instability (RIGI), which may develop as a result of genetic and epigenetic changes in parental germ cells following radiation exposure [62–64].

To provide evidence of RIGI in descendants and their parents, the frequency of chromosomal aberrations was analysed — their levels increase significantly in the presence of RIGI. In some cytogenetic studies, an elevated level of unstable and stable chromosomal aberrations in PBLs has been observed in descendants of irradiated individuals [16, 62, 65]. However, it should be noted that the dependence of cytogenetic parameters on the pre-conceptional radiation dose to parental gonads has not been investigated. Furthermore, interpretation of the results of cytogenetic studies in descendants living in contaminated areas is complicated by the inability to exclude the influence of their own in utero and postnatal radiation exposure.

Analysis of gene mutation frequency — which also increases significantly in the presence of RIGI — did not reveal an elevated mutation rate in the T-cell receptor gene among descendants of irradiated parents. In contrast, their parent-liquidators showed an increased mutation rate not only in the early period after radiation exposure, but also many years later [19, 20, 66]. Analysis of *de novo* mutations in children born after the accident to liquidators and residents of RCTs also failed to find evidence of a significant impact from pre-conceptional irradiation of parental gonads: up to 4,080 mGy in fathers and up to 550 mGy in mothers [67]. Thus, cytogenetic and molecular genetic studies do not allow us to conclude that transgenerational effects and RIGI are present in descendants of liquidators and children born after the accident who lived in RCTs.

Effects of in-utero radiation exposure. The extraordinary significance of research into the effects of in-utero radiation exposure is determined primarily by the high radiosensitivity of the embryo and fetus. An important component of radiation exposure after the accident was ¹³¹I, which entered the bodies of pregnant women through the alimentary route and affected the intrauterine development of the embryo and fetus. In isolated studies, genetic changes, physical development, and morbidity were assessed in children exposed to radiation during the antenatal period. For example, in individuals who underwent in-utero radiation exposure, a clear effect of increased frequency of blood lymphocytes TCR mutations was observed [68]. Moreover, the established patterns of somatic mutagenesis in in-utero exposed individuals persisted 28 years later [69].

In addition, disharmonious physical development was observed in children exposed to in-utero radiation, caused by an age-related increase in the proportion

⁸ Bolshakov LA, ed. Russian National Report: 35 years after the Chernobyl accident. results and prospects of overcoming its consequences in Russia. Moscow: Akadem-Print; 2021 (In Russ.).

of individuals with low body height [70]. However, the dose-response relationship for the radiation exposure effect on the fetus was not assessed.

Due to the significant inconsistency of global data, the incidence of leukemia in individuals exposed to radiation *in utero* was of considerable scientific interest. According to data from the National Radiation and Epidemiological Registry (NRER), no convincing evidence of an increased risk of leukemia development was obtained for children exposed to radiation *in utero* and in early childhood [71]. However, non-cancerous thyroid pathology (most commonly, diffuse non-toxic goiter) was diagnosed more frequently in these children than among non-exposed peers [72, 73]. The incidence rate in children exposed to radiation *in utero* increased sharply with age, reaching a maximum at 7–9 years, and by the age of 12 years decreased to the level observed in non-exposed peers [74, 75]. It is important to note that the observed children, after birth, lived in radiation-contaminated areas (RCA) and, due to consumption of breast milk and cow's milk, were also exposed to postnatal internal radiation. This factor was not taken into account in the study, nor was the level of iodine deficiency in the residential areas before and after the accident.

Thus, the conducted studies did not provide evidence of a link between the health status of children living in RCTc and *in utero* radiation exposure.

CONCLUSION

The analysis of data accumulated over 40 years of follow-up studies involving emergency workers, cleanup workers, and RCT residents has provided crucial new insights into both the early and long-term medical and biological effects of radiation exposure in humans. Among emergency workers, 134 cases of ARS were documented, including 28 fatal cases. New knowledge has been obtained about the pathogenesis, course, and outcomes of ARS in combination with radiation skin damage. The experience gained in diagnosing and treating ARS and its long-term consequences has led to a fundamental revision of clinical tactics and a significant improvement in treatment efficacy for this severe condition, which is characterized by high mortality.

No ARS cases were registered among the cleanup workers who participated in the emergency recovery operations after April 27, 1986, and the population residing in radioactively contaminated territories. The protective measures implemented after the accident (evacuation, establishment of a sanitary protection zone, etc.) significantly reduced radiation exposure levels among the population, remaining well below the threshold dose required to induce ARS.

A comprehensive radiobiological and radiation-epidemiological analysis revealed both preclinical changes, including cytogenetic, molecular genetics-based, and immunological markers, and clinically significant

alterations in the health condition of cleanup workers. Radiobiological studies have demonstrated persistently elevated levels of chromosomal aberrations in blood lymphocytes, mutations in genes encoding the T-cell receptor, impairment of T-cell immunity, and an elevated pro-inflammatory cytokine profile in serum.

Regular medical examinations of a large cohort of cleanup workers exposed to a wide range of radiation doses have provided crucial information on the radiation risk of the late effects of prolonged human exposure. A long-term radiation-epidemiological analysis shows that the incidence of leukemia in the first ten years after the accident exceeded the spontaneous level by 32%, and that of cancer and diseases of the cardiovascular system (primarily ischemic heart disease) over the 40-year follow-up period - by 8% and 5%, respectively. The research results support the scientific justification for including aplastic anemia and secondary immunodeficiency states (diseases of the blood and hematopoietic organs) in the Russian list of diseases eligible for inclusion in the list of radiation-related diseases.

Although an increase in the frequency of chromosomal aberrations in blood lymphocytes was observed in the exposed population of contaminated territories in the early period, a comprehensive assessment of the results from 40 years of health monitoring of the RCT population revealed no significant impact of radiation exposure on morbidity rates across all ICD-10 disease classes, except for TC.

According to the latest estimates by NRER specialists, conducted in early 2026, approximately 43% of thyroid cancer cases identified among exposed individuals who were under 18 years of age at the time of the accident are attributable to exposure to ^{131}I . The obtained evidence of the high carcinogenic radiosensitivity of the human thyroid gland to ^{131}I is of crucial importance for improving radiation protection measures for the population. For the first time, it has been demonstrated that chronic, predominantly internal thyroid irradiation in individuals who were children or adolescents at the time of the accident leads to an increased incidence of thyroid cancer 4–5 years after the event.

Analytical study results have established that the radiation risk of cancer from internal exposure to ^{131}I is comparable to the well-studied risk of thyroid cancer following external gamma radiation. However, quantitative estimates of the radiation risk of thyroid cancer remain rather uncertain and require further large-scale and in-depth research to obtain robust and statistically significant results. At the same time, a number of issues related to the TC induction in individuals exposed to radiation before the age of 18 remain relevant. To date, no molecular genetic markers of radiation-induced thyroid cancer have been identified.

It is a cause for concern that 40 years after the Chernobyl accident, an elevated radiation risk of thyroid cancer development still persists among people exposed to radiation during childhood and adolescence

in RCTs. In this context, it is extremely important to note that children represent a critical group in terms of thyroid radiation dose during such accidents. Timely implementation of preventive measures — such as early administration of stable iodine, replacement of locally produced cow's milk and meat — can prevent the development of thyroid cancer.

No evidence has been obtained of the impact of parental radiation exposure and *in utero* radiation exposure among RCTs residents on the health status of their descendants. In conclusion, it should be noted that the new radiobiological knowledge gained after the Chernobyl accident is of extremely high importance not only for radiobiology and radiation medicine. Estimates of adverse health effects are already being used today to improve global standards and ensure

radiation safety for both occupational workers and the general population.

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References

1. Piatkin EK, Nugis YuV, Chirkov AA. Estimation of absorbed dose based on the results of cytogenetic studies of lymphocyte cultures from subjects exposed in the accident at the Chernobyl Atomic Power Station. *Radiation Medicine*. 1989;34(6):52–7 (In Russ.).
2. Guskova AK, Galstyan IA, Gusev IA. *The accident on the Chernobyl nuclear power plant (1986–2011): health consequences, reflections of a physician*. Moscow: A.I. Burnasyan Federal Medical Biophysical Center of FMBA, Urals Research Center for Radiation Medicine; 2011 (In Russ.). EDN: [PCHCLR](#)
3. Rozhdestvenskiy LM, ed. *Sources, effects and risks of ionizing radiation. UNSCEAR 1988 Report to the General Assembly with scientific annexes*. Moscow: Mir; 1993 (In Russ.).
4. Baranov AE, Gale RP, Guskova AK, Piatkin EK, Chemplin R. Transplantation of the bone marrow after total body irradiation of the victims after the accident at the Chernobyl atomic power plant. *Hematology and Transfusiology*. 1989;3:3–16 (In Russ.). PMID: 2656374
5. Samoylov AS, Bushmanov AYU, Galstyan IA. Medical management: major lessons learned from the Chernobyl accident (the review). *Journal of Radiological Protection*. 2021;41(3):R51–60. <https://doi.org/10.1088/1361-6498/ac14d4>
6. Guskova AK, Baranov AE, Barabanova AV, Moiseev AA, Piatkin EK. The diagnosis, clinical picture and treatment of acute radiation sickness in the victims of the Chernobyl atomic electric power station. II. Non-bone marrow syndromes of radiation lesions and their treatment. *Therapeutic Archive*. 1989;61(8):99–103 (In Russ.).
7. *Health effects due to radiation from the Chernobyl accident. Annex D of UNSCEAR 2008: sources and effects of ionizing radiation*. New York: United Nations; 2012 (In Russ.).
8. Tsyb AF, Dedenkov AN, Ivanov VK, Stepanenko VF, Pozhidaev VV, et al. The development of an all-Union registry of persons exposed to radiation resulting from the accident at the Chernobyl atomic power station. *Medical Radiology*. 1989;34(7):3–6 (In Russ.). EDN: [VQKYOT](#)
9. Ivanov VK. *Liquidators: radiological consequences of Chernobyl*. Moscow: Centre of social and environmental initiatives of the nuclear industry; 2010 (In Russ.).
10. Bazyka D, Sushko V, Chumak A, Chumak V, Yanovych L, eds. *Health effects of the Chernobyl Accident Thirty Years Aftermath*. Kyiv: DIA; 2016.
11. Ivanov VK, Maksimov MA, Tumanov KA, Kochergina EV, Vlasov OK, Chekin SYu, et al. 35-year experience in the functioning of the National radiation and epidemiological registry as a state information system for monitoring the radiological consequences of the Chernobyl accident. *Radiation and Risk*. 2021;30(1):7–39 (In Russ.). <https://doi.org/10.21870/O131-3878-2021-30-1-7-39>
12. Pitkevich VA, Ivanov VK, Tsyb AF, Maksimov MA, Matyash VA, Shchukina NV. Dosimetric data of the all-Russia medical and dosimetric state registry for emergency workers. *Radiation and Risk*. 1995;S2:1–44 (In Russ.). EDN: [HVIRLP](#)
13. Schevchenko VA, Akayeva EA, Yeliseyeva IM, YelisoVA TV, Yofa EL, Nilova IN, et al. Human cytogenetic consequences of the Chernobyl accident. *Mutation Research*. 1996;361(1):29–34. [https://doi.org/10.1016/S0165-1161\(96\)90226-5](https://doi.org/10.1016/S0165-1161(96)90226-5)
14. Slozina N, Neronova E, Nikiforov A. Persistence of dicentric in Chernobyl clean-up workers who suffered from low doses of radiation. *Applied Radiation and Isotopes*. 2001;55(3):335–8. [https://doi.org/10.1016/S0969-8043\(01\)00059-8](https://doi.org/10.1016/S0969-8043(01)00059-8)
15. Snigiryova GP, Bogomazova AN, Novitskaya NN, Khazins ED. Results of the long-term cytogenetic monitoring of Chernobyl recovery workers. *Medical Radiology and Radiation Safety*. 2008;53(4):38–45 (In Russ.). EDN: [JTCYAP](#)
16. Vorobtsova IE, Semyonov AV. Complex cytogenetic characteristic of people suffered from Chernobyl accident. *Radiation Biology. Radioecology*. 2006;46(2):140–51 (In Russ.). EDN: [HTGIQN](#)
17. Snigiryova GP, Shevchenko VA, Novitskaya NN. The use of the FISH method for reconstructing the absorbed doses received by participants in the clean-up of the accident at the Chernobyl atomic electric power station. *Radiat Biol Radioecol*. 1995;35(5):654–61 (In Russ.). PMID: 7489098
18. Nugis VYu, Bushmanov AYU, Zapadinskaya HE, Kozlova MG, Tyhonova OA. Cytogenetic studies 28–29 years after the accident at the Chernobyl NPP. *Medical Radiology and*

- Radiation Safety*. 2016;61(4):35–42 (In Russ.).
EDN: [WHAWLH](#)
19. Smirnova SG, Orlova NV, Zamulaeva IA, Tkachenko NP, Lozebnoy NI, Kaplan MA, et al. Monitoring of the frequency of lymphocytes with mutations at T-cell receptor locus in Chernobyl cleanup workers. *Radiation and Risk*. 2012;21(1):20–9 (In Russ.).
EDN: [ORDUIR](#)
 20. Saenko AS, Zamulaeva IA, Smirnova SG, Orlova NV. Determination of somatic mutant frequencies at glycophorin A and T-cell receptor loci for biodosimetry of acute and prolonged irradiation. *Applied Radiation and Isotopes*. 2000;52(5):1145–8.
[https://doi.org/10.1016/S0969-8043\(00\)00061-0](https://doi.org/10.1016/S0969-8043(00)00061-0)
 21. Oradovskaya IV. *Immunological monitoring of the Chernobyl accident. Results of long term observations (1986–2000)*. Moscow: Knigizdat; 2021 (In Russ.).
 22. Yarin AA, Belyakov IM, Kusmenok OI, Arshinov VY, Simonova AV, Nadezhina NM, et al. Late T cell deficiency in victims of the Chernobyl radiation accident: possible mechanisms of induction. *International Journal of Radiation Biology*. 1993;63(4):519–28.
<https://doi.org/10.1080/09553009314550681>
 23. Kalinina NM, Davydova NI, Bychkova NV. Chernobyl liquidator's immune parameters in long period after accident (in 24 years). *Medico-Biological and Socio-Psychological Problems of Safety in Emergency Situations*. 2011;3:58–62 (In Russ.).
EDN: [QYKOTL](#)
 24. Pelevina II, Oradovskaya IV, Mansurova YuG, Aleschenko AV, Antoschina MM, Kudriashova OV, et al. The connection between molecular cellular parameters and immune status of liquidators after Chernobyl accident. *Radiation Biology. Radioecology*. 2010;50(5):501–7 (In Russ.).
EDN: [NBTCJP](#)
 25. Ivanov VK, Kashcheev VV, Karpenko SV, Glebova SE, Tumanov KA, Chekin SYu, et al. Leukemia incidence and mortality of recovery operation workers of the Chernobyl accident: assessment of radiation risks for the follow-up period 1986–2014. *Radiation Hygiene*. 2018;11(4):7–17 (In Russ.).
<https://doi.org/10.21514/1998-426X-2018-11-4-7-17>
 26. Ivanov VK, Tsyb AF, Khait SE, Kashcheev VV, Chekin SYu, Maksioutov MA, et al. Leukemia incidence in the Russian cohort of Chernobyl emergency workers. *Radiation and Environmental Biophysics*. 2012;51(2):143–9.
<https://doi.org/10.1007/s00411-011-0400-y>
 27. Kashcheev VV, Chekin SYu, Karpenko SV, Maksioutov MA, Tumanov KA, Kochergina EV, et al. Estimating of radiation risks of malignant neoplasms among Russian Chernobyl cleanup workers. *Radiation and Risk*. 2021;30(1):58–77 (In Russ.).
<https://doi.org/10.21870/0131-3878-2021-30-1-58-77>
 28. Ozasa K, Shimizu Yu, Suyama A, Kasagi F, Soda M, Grant EJ, et al. Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: An overview of cancer and noncancer diseases. *Radiation Research*. 2012;177(3):229–43.
<https://doi.org/10.1667/rr2629.1>
 29. Stewart FA, Akleyev AV, Hauer-Jensen M, Hendry LH, Kleiman NJ, MacVittie TJ, et al. ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs — threshold doses for tissue reactions in a radiation protection context. *Annals of ICRP*. 2012;41(1–2):1–322.
<https://doi.org/10.1016/j.icrp.2012.02.001>
 30. Chekin SYu, Gorski AI, Maksioutov MA, Korelo AM, Karpenko SV, Tumanov KA, et al. Impact of radiation in nerves system incidence among the workers involved in mitigation of the Chernobyl accident. *Radiation and Risk*. 2025;34(1):14–31 (In Russ.).
<https://doi.org/10.21870/0131-3878-2025-34-1-14-31>
 31. Chekin SYu, Maksioutov MA, Karpenko SV, Gorski AI, Tumanov KA, Shchukina NV, et al. Estimation of radiation risks of respiratory diseases in liquidators of the Chernobyl nuclear power plant accident consequences taking into account the influence of other diseases detected in them during the observation period. *Radiation and Risk*. 2025;34(2):21–38 (In Russ.).
<https://doi.org/10.21870/0131-3878-2025-34-2-21-38>
 32. Chekin SYu, Gorski AI, Maksioutov MA, Karpenko SV, Tumanov KA, Shchukina NV, et al. Assessment of radiation risks of digestive system diseases among Chernobyl liquidators, considering the influence of other diseases identified in them during the follow-up period. *Medical Radiology and Radiation Safety*. 2025;70(4):55–65 (In Russ.).
 33. Chekin SYu, Maksioutov MA, Kashcheev VV, Karpenko SV, Tumanov KA, Kochergina EV, et al. Estimating of radiation risks of non-cancer diseases among Russian Chernobyl cleanup workers. *Radiation and Risk*. 2021;30(1):78–93 (In Russ.).
<https://doi.org/10.21870/0131-3878-2021-30-1-78-93>
 34. Akleyev AA, Blinova EA, Akleyev AV. Single-nucleotide polymorphism as biomarkers of long-term radiation-induced changes in systemic immunity. *Pathogenesis*. 2021;19(3):38–49 (In Russ.).
<https://doi.org/10.25557/2310-0435.2021.03.38-49>
 35. Trapeznikova LN, Piskunov NF. Experience of radiation-hygienic monitoring management and assessment of radiation situation in the Bryansk territory after 25 years since the day of the Chernobyl accident. *Radiation Hygiene*. 2011;4(2):113–9 (In Russ.).
EDN: [NXLBUB](#)
 36. Menyajlo AN, Kashcheev VV, Pryakhin EA, Maksyutov MA, Tumanov KA, et al. Forecast of Radiation risks of the population in the contaminated ¹³⁷Cs territories of Russia, in accordance with current ICRP recommendations. *Medical Radiology and Radiation Safety*. 2020;65(3):45–52 (In Russ.).
<https://doi.org/10.12737/1024-6177-2020-65-3-45-52>
 37. Shinkarev S, Voillequé P, Gavrillin Yu, Khrouch V, Bouville A, Hoshi M, et al. Credibility of Chernobyl thyroid doses exceeding 10 Gy based on in-vivo measurements of ¹³¹I in Belarus. *Health Physics*. 2008;94(2):180–7.
<https://doi.org/10.1097/01.HP.0000288044.73410.6b>
 38. Onishchenko GG, Popova AYu, eds. *Radiological and hygienic issues of the mitigation of the Chernobyl NPP accident consequences*. St.-Petersburg: Saint-Petersburg Research Institute of Radiation Hygiene after Professor P.V. Ramzaev; 2016 (In Russ.).
 39. Vorobtsova IE, Kolyubaeva SN, Vorob'eva MV, Korotkov DV, Komar VE. Cytogenetic characteristics of children who suffered as a result of the Chernobyl power plant accident. *Medical Radiology*. 1993;38(10):25–8 (In Russ.).
EDN: [KSQMAQ](#)
 40. Vorobtsova IE, Bogomazova AN. Stable chromosome aberrations in the lymphocytes of the peripheral blood in persons who suffered as a result of the accident at the Chernobyl atomic electric power station. *Radiation Biology. Radioecology*. 1995;35(5):636–40 (In Russ.).
PMID: 7489095

41. Shevchenko VA, Snigiryova GP. The significance of cytogenetic investigation for the estimation of Chernobyl accident consequences. *Radiation Biology. Radioecology*. 2006;46(2):133–9 (In Russ.).
EDN: [HTGIQD](#)
42. Zupunski L, Ostroumova E, Drozdovitch V, Veyalkin I, Ivanov V, Yamashita S, et al. Thyroid cancer after exposure to radioiodine in childhood and adolescence: 131I-related risk and the role of selected host and environmental factors. *Cancers*. 2019;11(10):1481.
<https://doi.org/10.3390/cancers11101481>
43. Ivanov VK, Kashcheev VV, Chekin SYu, Maksoutov MA, Tumanov KA, Kochergina EV, et al. Assessment of radiation risks of malignant neoplasms among the population of Russian regions contaminated with radionuclides as a result of the Chernobyl accident. *Radiation and Risk*. 2021;30(1):131–46 (In Russ.).
<https://doi.org/10.21870/0131-3878-2021-30-1-131-146>
44. Brenner AV, Mykola DT, Hatch M, Bogdanova TI, Oliynik VA, Lubin JH, et al. I-131 Dose response for incident thyroid cancers in Ukraine related to the Chernobyl accident. *Environmental Health Perspectives*. 2011;119(7):933–9.
EDN: [OLBVRP](#)
45. Zablotska LB, Ron E, Rozhko AV, Hatch M, Polyanskaya ON, Brenner AV, et al. Thyroid cancer risk in Belarus among children and adolescents exposed to radioiodine after the Chernobyl accident. *British Journal of Cancer*. 2011;104(1):181–7.
<https://doi.org/10.1038/sj.bjc.6605967>
46. Mikhailova AA, Shestakov AV, Chubakova KA, Kolokolova EV, Eliseev VY, Kostyaeva MY, et al. Modern concepts of the molecular pathogenesis of thyroid cancer. *Advances in Molecular Oncology*. 2021;8(2):8–22 (In Russ.).
<https://doi.org/10.17650/2313-805X-2021-8-2-8-22>
47. Kachko VA, Platonova NM, Vanushko VE, Shifman BM. The role of molecular testing in thyroid tumors. *Problems of Endocrinology*. 2020;66(3):33–46 (In Russ.).
<https://doi.org/10.14341/prob12491>
48. Yakushina VD, Lerner LV, Kazubskaya TP, Kondrat'ieva TT, Subramanian S, Lavrov AV. Molecular genetics of follicular cell thyroid carcinoma. *Clinical and Experimental Thyroidology*. 2016;12(2):55–64 (In Russ.).
<https://doi.org/10.14341/ket2016255-64>
49. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nature Reviews Endocrinology*. 2011;7(10):569–80.
<https://doi.org/10.1038/nrendo.2011.142>
50. Lushnikov EF, Tsyb AF, Yamashita S. *Thyroid cancer in Russia after the Chernobyl*. Moscow: Medicine; 2006 (In Russ.).
51. Menicali E, Moretti S, Voce P, Romagnoli S, Avenia N, Puxeddu E. Intracellular signal transduction and modification of the tumor microenvironment induced by RET/PTCs in papillary thyroid carcinoma. *Frontiers in Endocrinology*. 2012;3:67.
<https://doi.org/10.3389/fendo.2012.00067>
52. Thomas G. Radiation and thyroid cancer—an overview. *Radiation Protection Dosimetry*. 2018;182(1):53–7.
<https://doi.org/10.1093/rpd/ncy146>
53. Nikiforov YE. Is ionizing radiation responsible for the increasing incidence of thyroid cancer? *Cancer*. 2010;116:1626–8.
<https://doi.org/10.1002/cncr.24889>
54. Biryukov AP, Ushenkova LN, Koterov AN. RET/PTC gene rearrangements in children's papillary thyroid carcinoma after the Chernobyl accident: evidence of tumors incomplete radiation attributiveness. *Medical and Biological Problems of Life Activity*. 2015;2(14):24–40 (In Russ.).
EDN: [WJBQZL](#)
55. Rogova MO, Novosad SV, Martirosian NS, Trukhina LV, Petunina NA. Molecular markers as risk factors for thyroid cancer. *Therapeutic Archive*. 2019;91(10):119–23 (In Russ.).
<https://doi.org/10.26442/00403660.2019.10.000357>
56. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. *Nature Reviews Cancer*. 2013;13(3):184–99.
<https://doi.org/10.1038/nrc3431>
57. Lima J, Trovisco V, Soares P, Máximo V, Magalhães J, Salvatore G, et al. BRAF mutations are not a major event in post-Chernobyl childhood thyroid carcinomas. *Journal of Clinical Endocrinology and Metabolism*. 2004;89(9):4267–71.
<https://doi.org/10.1210/jc.2003-032224>
58. Kochergina EV, Gorski AI, Chekin SYu, Korelo AM, Tumanov KA, Zelenskaya NS, et al. General morbidity among children of Chernobyl cleanup workers: radiation epidemiological study. *Radiation and Risk*. 2021;30(1):110–30 (In Russ.).
<https://doi.org/10.21870/0131-3878-2021-30-1-110-130>
59. Korenev NM, Borisko GA, Kashina-Yarmak VL. Health status of children, born in families of parents which were irradiated due to Chernobyl NPP accident. *Pediatric Health*. 2012;6:66–70 (In Russ.).
EDN: [PFXMEZ](#)
60. Liaginskaya AM, Tukov AR, Osipov VA, Ermalitskiy AP, Prokhorova ON. Congenital malformations among offspring of the liquidators of the consequences from Chernobyl accident. *Radiation Biology. Radioecology*. 2009;49(6):694–702.
EDN: [KYGPVD](#)
61. Koterov AN, Biryukov AP. The offspring of liquidators of Chernobyl atomic power station accident 1. The estimation of the basic opportunity to register of radiation effects. *Medical Radiology and Radiation Safety*. 2012;57(1):58–79 (In Russ.).
EDN: [SNYBRL](#)
62. Aghajanyan AV, Suskov II. Genomic instability in children born after the Chernobyl nuclear accident (*in vivo* and *in vitro* studies). *Russian Journal of Genetics*. 2010;46(6):740–9.
<https://doi.org/10.1134/S1022795410060153>
63. Suskov II, Kuzmina NS, Suskova VS, Baleva LS, Sipyagina AE. The problem of induced genome instability as the basis of increased morbidity among children exposed to low-intensity radiation at low doses. *Radiation Biology. Radioecology*. 2006;46(2):167–77 (In Russ.).
EDN: [HTGIRR](#)
64. Baleva LS, Sukhorukov VS, Sipyagina AE, Karakhan NM, Voronkova AS, Sadykov AR. The role of genomic instability and expression of the p53 protein gene network in the processes of oncogenesis in first- and second-generation children living in radioactively contaminated areas. *Russian Bulletin of Perinatology and Pediatrics*. 2017;62(1):81–6 (In Russ.).
<https://doi.org/10.21508/1027-4065-2017-62-1-81-86>
65. Aghajanyan A, Suskov I. Transgenerational genomic instability in children of irradiated parents as a result of the Chernobyl nuclear accident. *Mutation Research*. 2009;671(1-2):52–7.
<https://doi.org/10.1016/j.mrfmmm.2009.08.012>
66. Baleva LS, Sipyagina AE, Smirnova SG, Selivanova EI, Kuzmina TB, Orlova NV, et al. Value of studying the frequency of mutant cells from T-cell receptor loci in Chernobyl accident liquidator's children in forming high carcinogenic risk groups. *Russian Bulletin of Perinatology and Pediatrics*.

- 2010;55(4):102–9 (In Russ.).
EDN: [MVVAHX](#)
67. Yeager M, Machiela MJ, Kothiyal P, Dean M, Bodelon C, Suman S, et al. Lack of transgenerational effects of ionizing radiation exposure from the Chernobyl accident. *Science*. 2021;372(6543):725–9.
<https://doi.org/10.1126/science.abg2365>
 68. Zamulaeva IA, Smirnova SG, Orlova NV, Vereschagina AO, Chekin SYu, Smirnova IA, et al. Somatic mutagenesis at T-cell receptor locus in inhabitants of radiation polluted regions as a result of the Chernobyl disaster. *Radiation Biology. Radioecology*. 2006;46(3):307–14 (In Russ.).
EDN: [HTVIRR](#)
 69. Smirnova SG, Orlova NV, Krikunova LI, Mkrtchyan LS, Zamulaeva IA. Frequency of lymphocytes with mutations at the locus of T-cell receptor in resident of radiation polluted Bryansk region after 28 years after the Chernobyl nuclear power plant accident. *Radiation Biology. Radioecology*. 2016;56(3):285–92 (In Russ.).
<https://doi.org/10.7868/S0869803116030164>
 70. Gorobets VF, Davydov GA, Gorobets NYa, Davydova EV. Effects of prenatal exposure to the Chernobyl radioiodine on postnatal physical development of children from the Kaluga region. *Radiation and Risk*. 2020;29:129–39 (In Russ.).
<https://doi.org/10.21870/0131-3878-2020-29-1-129-139>
 71. Davis S, Day RW, Kopecky KJ, Mahoney MC, McCarthy PL, Michalek AM, et al. Childhood leukaemia in Belarus, Russia, and Ukraine following the Chernobyl power station accident: results from an international collaborative population-based case-control study. *International Journal of Epidemiology*. 2006;35(2):386–96.
<https://doi.org/10.1093/ije/dyi220>
 72. Tsyb AF, Matveenko EG, Gorobets VF, Borovikova MP, Temnikova EI, Gorobets NYa. Dynamics of the morphological and functional state of thyroid in utero irradiated children from the south-west part of the Kaluga region during the first ten years after the Chernobyl accident. *Radiation and Risk*. 2001;12:42–7 (In Russ.).
EDN: [IJTMFL](#)
 73. Gorobets VF. Incidence of thyroid diseases in the period before puberty at the Kaluga oblast children irradiated owing to technogenic incorporation ¹³¹I on neonatal and early breast-feeding stage. *Medical and Biological Problems of Life Activity*. 2012;1(7):11–7 (In Russ.).
EDN: [PJBFGN](#)
 74. Gorobets VF. The dynamics of thyroid diseases incidence in the period before puberty at children from southwest region of the Kaluga oblast irradiated in utero owing to technogenic iodine-131 incorporation. *Radiation and Risk*. 2012;21(1):30–8 (In Russ.).
EDN: [ORDUJL](#)
 75. Gorobets VF. Analyses of dynamics of thyroid diseases incidence in the period before puberty at the Kaluga region children irradiated owing to technogenic ¹³¹I incorporation on antenatal, neonatal and breast-feeding stages of development. *Medical and Biological Problems of Life Activity*. 2013;2(10):109–16 (In Russ.).

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DEVELOPMENT OF CRITERIA FOR TRIAGE OF EXPOSED INDIVIDUALS BASED ON DICENTRIC CHROMOSOME ANALYSIS: A PILOT STUDY

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Introduction. In the event of mass radiation incidents, medical triage of exposed individuals must enable rapid identification of individuals at risk of developing the hematopoietic form of acute radiation sickness (ARS).

Objective. Development of effective cytogenetic criteria for identifying individuals at increased risk of developing the hematopoietic form of ARS during mass radiation incidents.

Materials and methods. The study involved 12 donors aged 23–73 years. The study object was phytohemagglutinin-stimulated T-lymphocytes from peripheral blood. Blood samples were exposed to *in vitro* gamma-irradiation at doses of 1 Gy and 2 Gy using an IGUR-1M unit. Cytogenetic preparations were obtained according to a standardized cytogenetic protocol and stained with a 2% Giemsa solution. Images were digitized and analyzed using the Metafer/Ikaros (Metasystems, Germany) software package. The frequency of chromosomal aberrations per cell was assessed. Metaphases of T-lymphocytes with dicentric chromosomes were counted in the order of their occurrence during slide analysis. For statistical analysis, Past 4.01 and SPSS Statistics 21 software packages were used.

Results. The decision regarding the classification of a potentially irradiated sample as one falling into the dose range likely to cause ARS development should be based on an analysis of the number of T-lymphocyte metaphases required to identify five dicentric chromosomes. In the study, dicentric chromosomes were detected in samples without irradiation in 33% of the examined individuals. After *in vitro* irradiation of blood samples at a dose of 1 Gy, the average frequency of dicentric chromosomes was 0.073 ± 0.008 per cell, reaching 0.28 ± 0.02 per cell at a dose of 2 Gy.

Conclusions. A preliminary algorithm for differentiating *in vitro* irradiated cytogenetic samples into dose ranges has been developed. The identification of the fifth dicentric chromosome in order of analysis within the first 26 T-lymphocyte metaphases served as the basis for assigning the sample to a dose range of 2 Gy and above. In cases where the fifth dicentric chromosome was identified between the 27th and 85th metaphase, the sample was assigned to a dose range of 1 Gy and above. In cases where fewer than five dicentric chromosomes were detected upon analysis of 85 metaphases, the sample was assigned to a dose range below 1 Gy. Future research will be aimed at refining the algorithm and validating the results.

Keywords: dicentric chromosome; chromosomal aberrations; cytogenetic analysis; medical triage; gamma-irradiation; acute radiation sickness

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Compliance with the ethical principles: informed consent for blood sample collection and further cytogenetic studies was obtained from all participants. The study was approved by the Ethics Committee of the Southern Urals Federal Research and Clinical Center for Medical Biophysics (Minutes No. 9 dated 20.11.2023).

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РАЗРАБОТКА КРИТЕРИЕВ ДЛЯ СОРТИРОВКИ ОБЛУЧЕННЫХ ЛИЦ НА ОСНОВЕ АНАЛИЗА ДИЦЕНТРИЧЕСКИХ ХРОМОСОМ: ПИЛОТНОЕ ИССЛЕДОВАНИЕ

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Введение. При массовых радиационных инцидентах для медицинской сортировки облученных лиц необходимо иметь подход, позволяющий оперативно определить группу риска по развитию костномозговой формы острой лучевой болезни (ОЛБ).

Цель. Разработка эффективных цитогенетических критериев для выявления людей с повышенным риском развития костномозговой формы ОЛБ при массовых радиационных инцидентах.

Материалы и методы. В исследовании приняли участие 12 доноров в возрасте 23–73 лет. Объектом исследования были стимулированные фитогемагглютинином Т-лимфоциты периферической крови. Образцы крови подвергали *in vitro* гамма-облучению в дозах 1 и 2 Гр с использованием установки ИГУР-1М. Цитогенетические препараты получали по стандартизированному

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цитогенетическому протоколу и окрашивали 2% раствором Гимзы. Изображения оцифровывали и анализировали с помощью Metafer/Ikaros (Metasystems, Германия). Оценивали частоту хромосомных aberrаций на одну клетку. Проводили подсчет метафаз Т-лимфоцитов с дицентрическими хромосомами по порядку их встречаемости при анализе препарата. Для статистического анализа использовали программы Past 4.01 и SPSS Statistics 21.

Результаты. Для принятия решения об отнесении потенциально облученного образца к дозовому диапазону, при котором вероятно развитие ОЛБ, необходимо проанализировать то количество метафаз Т-лимфоцитов, в которых будет идентифицировано пять дицентрических хромосом. В ходе исследования в образцах без облучения дицентрические хромосомы были выявлены у 33% обследуемых лиц. После *in vitro* облучения образцов крови в дозе 1 Гр средняя частота встречаемости дицентрических хромосом составила $0,073 \pm 0,008$ на одну клетку, в дозе 2 Гр — $0,28 \pm 0,02$ на одну клетку.

Выводы. Разработан предварительный алгоритм разделения *in vitro* облученных цитогенетических образцов на дозовые диапазоны. Идентификация пятой дицентрической хромосомы по порядку анализа в первых 26 метафазах Т-лимфоцитов являлась основанием для отнесения исследуемого образца к дозовому диапазону от 2 Гр и выше. Если пятая дицентрическая хромосома была выявлена с 27-й по 85-ю метафазу, образец относили к дозовому диапазону от 1 Гр и выше. Если при анализе 85 метафаз было выявлено менее пяти дицентрических хромосом, образец относили к дозовому диапазону менее 1 Гр. Планируется продолжение исследований с доработкой алгоритма и валидацией результатов.

Ключевые слова: дицентрическая хромосома; хромосомные aberrации; цитогенетический анализ; медицинская сортировка; гамма-облучение; острая лучевая болезнь

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Соответствие принципам этики: от всех участников получено информированное согласие на забор образцов крови и дальнейшие цитогенетические исследования. Исследование одобрено этическим комитетом ФГБУН УНПЦРМ ФМБА России (протокол № 9 от 20.11.2023).

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INTRODUCTION

In the event of large-scale radiation incidents involving a large number of people, physical methods for dose reconstruction may be difficult or impossible to apply. In incidents involving the public, the lack of dose information complicates accurate assessment of the risks of medical and biological effects of radiation, thus impeding the decision-making process [1, 2].

Prompt identification of individuals with a high probability of developing moderate to severe acute radiation sickness (ARS) is essential for their timely referral to specialized facilities. The hematopoietic form of ARS is a treatable form, which occurs at radiation doses ranging 1–10 Gy, with varying degrees of disease severity classified within this range depending on the dose. According to the literature data, clinically recognizable signs of the hematopoietic syndrome of ARS are noted after exposure at doses above 1 Gy. In the exposure dose range of 1–2 Gy, mild ARS is observed, while moderate ARS is observed in the dose range of 2–4 Gy [3, 4].

As a rule, medical assessment includes taking a history (including location during the incident), physical

examination, documenting clinical symptoms, and, when possible, determining the absolute lymphocyte and/or neutrophil count. Although hematological investigations are helpful in assigning exposed individuals into critical groups, they yield ambiguous results in cases of combined trauma or comorbid conditions [3, 5–7].

Assessment of radiation dose is critical for planning medical care. When physical dosimetry is impossible, cytogenetic biodosimetry based on the scoring of unstable chromosomal aberrations in metaphases of peripheral blood T-lymphocytes can be applied [6, 8]. Elevated frequencies of dicentric chromosomes and centric rings in the spectrum of observed aberrations indicate exposure to ionizing radiation. The analysis of these types of chromosomal aberrations is referred to as the “gold standard” in biological dosimetry, due to the established dose-effect relationship and the low spontaneous frequency of these aberration types [9]. The cytogenetic method requires specialized laboratory conditions, where cell culture is performed for approximately two days, followed by slide preparation and staining, microscopic analysis with image digitization, and dose calculation using calibration curves [10–13]. Manually, one researcher can analyze no more than ~500 cells per

working day, rendering this method labor- and resource-intensive.

In mass casualty incidents, the goal of rapid medical triage requires approaches that significantly reduce resource intensity and increase laboratory throughput, rather than precise estimation of absorbed radiation doses. Therefore, in this study, we set out to create an approach for rapid cytogenetic examination of exposed individuals, who had undergone relatively uniform whole-body gamma-exposure, and identification of those at risk of developing the hematopoietic syndrome of ARS.

Our research is based on the hypothesis about the possibility of classifying *in vitro* gamma-irradiated blood samples into dose ranges corresponding to the development of mild and moderate severity ARS. This can be carried out by detecting unstable chromosomal aberrations during the analysis of a relatively small number of peripheral blood T-lymphocyte metaphases. It is assumed that an increase in the gamma-irradiation dose is associated with an increase in the probability of detecting dicentric chromosomes in the earliest analyzed T-lymphocyte metaphases. The proposed approach is based on automated slide digitization and data analysis using specialized software, which ensures higher quality of the primary material obtained, reproducibility of results, and allows researchers to verify the detected chromosomal aberrations.

In this work, we aim to develop effective cytogenetic criteria for identifying individuals at increased risk of developing the hematopoietic form of ARS in mass radiation incidents.

MATERIALS AND METHODS

The study involved 12 donors:

- 7 persons aged 57–73 years (mean age — 60 ± 2 years), 5 female and 2 male donors;
- 5 persons aged 23–38 years (mean age 27 ± 3 years), 4 female and 1 male donors.

At the time of blood sample collection, the donors had no history of treatment for diseases using ionizing radiation sources. There were also no severe forms of diabetes mellitus, autoimmune diseases, history of blood transfusion, and radiological examinations in the three months preceding the study.

To identify individuals meeting the above criteria, the potential participants underwent a preliminary interview. In the absence of exclusion criteria, a questionnaire was administered, and consent for the processing of personal data and participation in the study was obtained.

Obtaining metaphase chromosomes from human peripheral blood T-lymphocytes

Venous blood, 6 mL in volume, was collected from the cubital vein into sterile heparinized tubes. For

each individual, three samples were prepared: two of them were irradiated *in vitro* on an IGUR-1M unit (Kvant, Russia) at doses of 1 Gy and 2 Gy, while the third sample was not irradiated. The IGUR-1M has the following characteristics: ^{137}Cs source, dose rate of 0.014 Gy/s, and irradiation non-uniformity of 5%.

Cytogenetic preparations from peripheral blood T-lymphocytes were obtained according to a protocol that included four sequential stages: cell culturing to the metaphase stage, hypotonic treatment, fixation of metaphase plates, and preparation of chromosome spreads, as described in [14].

T-cell culturing was conducted in sterile vacuum containers (Medpolymer, Russia). The culture consisted of 2 mL of blood, 5 mL of RPMI-1640 medium (PanEco, Russia), 0.5 mL of qualified fetal bovine serum (PanEco, Russia), and phytohemagglutinin (PHA) at a final concentration of 20 mg/mL (PanEco, Russia). Cells were cultured in a CO_2 incubator at 37.5°C for 54 h. Two hours before the end of culturing, a colchicine solution (PanEco, Russia) was added to the culture at a final concentration of 0.03 mg/mL.

For further processing, the cell suspension was transferred to centrifuge tubes and centrifuged at 1500 rpm for 10 min in a centrifuge (ThermoScientific, USA). The supernatant was removed, leaving the pellet. For hypotonic treatment, warm (37°C) 0.55% KCl solution was added, the pellet was resuspended, and the mixture was incubated in a thermostat at 37°C for 30 min. The mixture was then centrifuged for 10 min at 1100 rpm. The supernatant was carefully removed, the pellet was resuspended, and cold (4°C) freshly prepared fixative liquid (ethanol to glacial acetic acid in the ratio of 3:1) was added, bringing the total volume in the tube to 10 mL. The cells were left in the fixative medium for 10 min at 4°C, then centrifuged at 1500 rpm for 10 min. The supernatant was removed, and a fresh portion of fixative agent was added. Thus, the cells were passed through the fixative agent three times.

To obtain chromosome spreads, the total volume of the pellet in the tube was adjusted to 1.5 mL with a fresh fixative liquid, mixed well with a pipette, and the cell suspension was dropped onto a chilled slide (two drops). The slide was dried on a heating plate at 40°C. The quality of the preparation was checked using phase-contrast microscopy. Three slides were prepared per donor. The slides were stained with 2% Giemsa stain (PanEco, Russia) for 5–6 min.

Analysis of chromosome preparations was conducted using light microscopy. To that end, images of high-quality metaphases were obtained using the Metafer scanning and imaging platform (Metasystems, Germany) with a $\times 63$ immersion objective. Image processing was performed using the Ikaros software (Metasystems, Germany).

For cytogenetic analysis, metaphase plates containing 45–47 chromosomes, with good spreading and no overlaps, were selected. During the study, a cytogenetic protocol was completed, recording the following chromosomal aberrations: dicentric chromosomes, ring chromosomes, paired fragments, and acentric rings. For each slide, 500–1000 cells were scored in unirradiated samples and 220–500 cells in irradiated samples. The figure presents images of T-lymphocyte metaphases with chromosomal aberrations.

The identified chromosomal aberrations were grouped as follows: dicentric chromosomes, unstable chromosomal exchanges (dicentric and ring chromosomes), paired fragments, acentric rings.

The work involved counting T-lymphocyte metaphases with dicentric chromosomes in the order of their occurrence among all metaphases included in the analysis on a digitized slide.

Methods for statistical data processing

The frequency of chromosomal aberrations was calculated per cell. Standard descriptive statistical methods were used: mean values and the standard error of the mean were calculated for chromosomal aberrations.

When calculating the number of T-lymphocyte metaphases in which dicentric chromosomes were identified in the order of their occurrence during slide analysis, the data are presented as the median (M_0) and the 95% confidence interval (CI). The CI for the median estimate was calculated using the bootstrap procedure with

1000 iterations. The 10th and 90th percentiles (%), as well as the minimum and maximum individual values for the number of T-lymphocytes with dicentric chromosomes in the order of their occurrence during slide analysis, were also assessed.

The expected number of cells in which dicentric chromosomes can potentially be identified (D_n) was calculated using formula (1):

$$X = \frac{D_n}{F}, \quad (1)$$

wherein X — the expected number of cells in which dicentric chromosomes can be identified in the order of their occurrence on the slide; D_n — sequence number of dicentric chromosomes during slide analysis ($D_n = 1, 2, 3, 4, 5, 10, 15, 20, 25, 30$); F — frequency of dicentric chromosomes, calculated per cell.

To calculate the probability of encountering dicentric chromosomes in unirradiated samples for each donor, the average number of dicentric chromosomes was analyzed using the moving average method with a window size of 10, 20, 30, 50, and 100 cells. Subsequently, based on these values, the probability of the frequency of dicentric chromosomes was calculated using formula (2):

$$P = \frac{m}{k} \times 100\%, \quad (2)$$

wherein P — probability of dicentric chromosome occurrence; m — number of identified dicentric chromosomes; k — number of analyzed cells (window size).

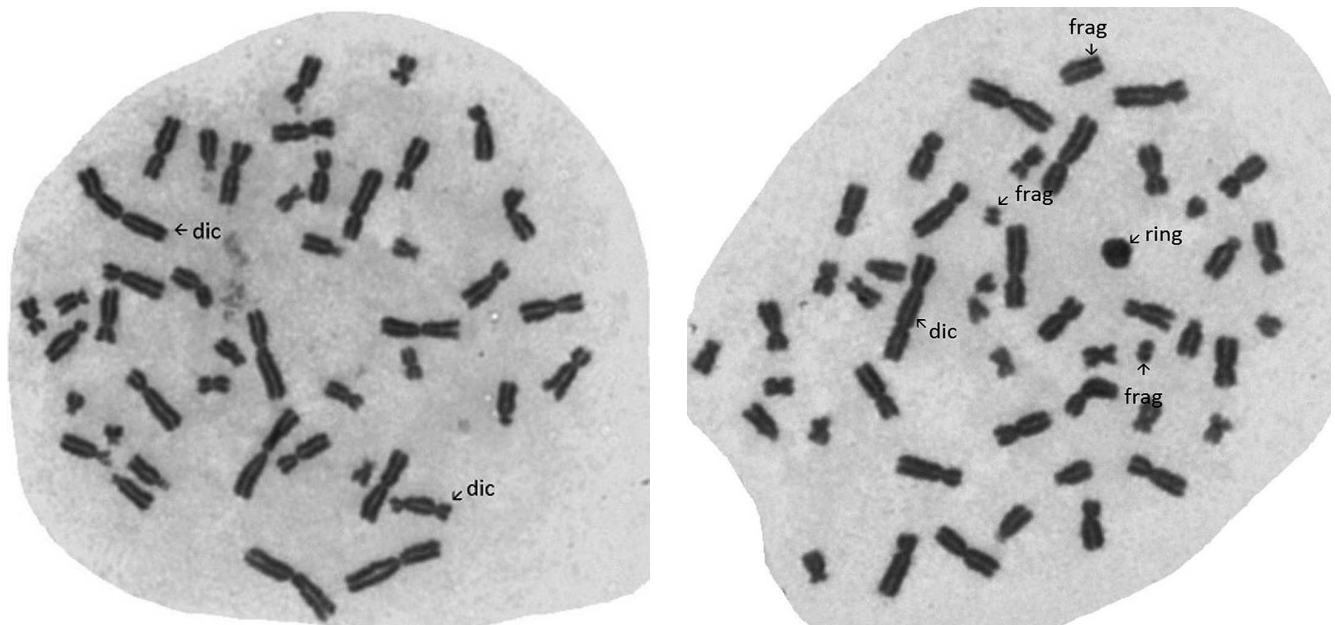


Figure prepared by the authors based on their own data

Fig. T-lymphocyte metaphases with chromosomal aberrations: dic — dicentric chromosome; frag — paired fragment; ring — ring chromosome; Images of T-lymphocyte metaphases were obtained using the Ikaros software (immersion objective, magn. $\times 63$)

For statistical analysis, the Past 4.01 and SPSS Statistics 21 statistical software were used.

RESULTS AND DISCUSSION

During the study, dicentric chromosomes were detected in 33% of the examined individuals (4 out of 12) in unirradiated samples. The frequency of dicentric chromosomes ranged 0–1 abs. unit per 500 cells and 0–2 abs. units per 1000 cells. The mean frequency of dicentric chromosomes and unstable chromosomal exchanges was 0.0007 ± 0.0003 per cell, of paired fragments — 0.016 ± 0.003 per cell, and of acentric rings — 0.0003 ± 0.0002 per cell.

After *in vitro* irradiation of blood samples at a dose of 1 Gy, the mean frequency of chromosomal aberrations increased statistically significantly compared to unirradiated samples ($p < 0.0001$). The mean frequency of dicentric chromosomes was 0.073 ± 0.008 per cell, unstable chromosomal exchanges — 0.081 ± 0.009 per cell, paired fragments — 0.17 ± 0.01 per cell, and acentric rings — 0.009 ± 0.002 per cell.

For *in vitro* irradiation at a dose of 2 Gy, the mean frequency of dicentric chromosomes was 0.28 ± 0.02 per cell, unstable chromosomal exchanges — 0.3 ± 0.02 per cell, paired fragments — 0.64 ± 0.03 per cell, and acentric rings — 0.03 ± 0.005 per cell (Table 1).

In unirradiated samples (among those individuals in whom dicentric chromosomes were detected), the expected number of T-lymphocytes with one dicentric chromosome ranged 412–500 cells. The first dicentric chromosome was identified in T-lymphocytes with sequence numbers 77, 169, 341, and 314. The second dicentric chromosome was identified in two donors: in a T-lymphocyte with sequence number 341 (one cell contained two dicentric chromosomes) and in cell number 492.

Table 2 presents the expected number of cells in which dicentric chromosomes can be identified in the order of cell scoring following *in vitro* irradiation at doses of 1 Gy and 2 Gy. According to the presented data, after irradiation at a dose of 1 Gy, two dicentric chromosomes were detected in T-lymphocytes between the 17th and 69th cells scored, $M_e = 27$ [95% CI: 21; 41]. To detect 5 dicentric chromosomes, it was necessary to analyze 67 T-lymphocytes [95% CI: 41; 83]; for 10 dicentric chromosomes — 135 T-lymphocytes [95% CI: 103; 206]; for 20 dicentric chromosomes — 270 T-lymphocytes [95% CI: 206; 414]; and to identify 30 dicentric chromosomes — 405 T-lymphocytes [95% CI: 309; 620].

At an *in vitro* irradiation dose of 2 Gy, the expected number of T-lymphocytes that need to be analyzed to detect a comparable number of dicentric chromosomes was statistically significantly lower than that at an *in vitro* irradiation dose of 1 Gy ($p < 0.001$): 2 dicentric chromosomes were detected between the 5th and 12th cells; the median value for T-lymphocytes was 8 [95% CI: 6; 9]; 5 dicentric chromosomes were detected upon analysis of 19 T-lymphocytes [95% CI: 16; 22]; 10 dicentric chromosomes — upon analysis of 38 T-lymphocytes [95% CI: 31; 45]; 20 dicentric chromosomes — upon analysis of 77 T-lymphocytes [95% CI: 63; 90]; and 30 dicentric chromosomes — upon analysis of 115 T-lymphocytes [95% CI: 94; 134].

Table 3 presents data on the number of T-lymphocyte metaphases with detected dicentric chromosomes in the order of their analysis on digitized cytogenetic slides. Upon *in vitro* irradiation at a dose of 1 Gy, the median number of cells in which dicentric chromosomes were detected differed statistically significantly from the expected values only for the 1st, 25th, and 30th dicentric chromosomes in the order of their identification during analysis. For instance, the expected median value for T-lymphocytes was statistically significantly ($p = 0.002$)

Table 1. Mean frequency of chromosomal aberrations under *in vitro* irradiation (per cell)

Type of chromosomal aberrations	Mean frequency of chromosomal aberrations under <i>in vitro</i> irradiation, per cell		
	Irradiation dose		
	unirradiated	1 Gy	2 Gy
Dicentric chromosomes	$0.0007 \pm 0.0003^*$	$0.073 \pm 0.008^{**}$	0.28 ± 0.02
Ring chromosomes	0^*	$0.008 \pm 0.002^{**}$	0.02 ± 0.006
Unstable chromosomal exchanges	$0.0007 \pm 0.0003^*$	$0.081 \pm 0.009^{**}$	0.3 ± 0.02
Paired fragments	$0.016 \pm 0.003^*$	$0.17 \pm 0.01^{**}$	0.64 ± 0.03
Acentric rings	$0.0003 \pm 0.0002^*$	$0.009 \pm 0.002^{**}$	0.03 ± 0.005

Table compiled by the authors based on their own data

Note: Data are presented as the mean value and standard error of the mean; * — statistically significant differences in the frequency of chromosomal aberrations between unirradiated and irradiated samples, $p < 0.0001$; ** — statistically significant differences in the frequency of chromosomal aberrations between samples with *in vitro* irradiation at a dose of 1 Gy and samples with *in vitro* irradiation at a dose of 2 Gy, $p < 0.05$.

Table 2. Expected number of cells in which dicentric chromosomes can be identified

Number of dicentric chromosomes	Irradiation dose					
	1 Gy			2 Gy		
	Number of T-lymphocyte metaphases			Number of T-lymphocyte metaphases		
	Median [CI]	10–90 percentiles	min–max	Median [CI]	10–90 percentiles	min–max
1	13* [10–21]	9–32	9–34	4 [3–4]	3–5	3–6
2	27* [21–41]	18–64	17–69	8 [6–9]	5–11	5–12
3	40* [31–62]	27–96	26–103	12 [9–13]	8–16	8–17
4	54* [41–83]	36–129	34–138	15 [13–18]	10–22	10–23
5	67* [52–103]	45–161	43–172	19 [16–22]	13–27	13–29
10	135* [103–206]	91–321	86–344	38 [31–45]	25–56	25–58
15	202* [155–310]	136–482	129–517	58 [47–67]	38–82	38–87
20	270* [206–414]	182–643	172–689	77 [63–90]	50–110	50–116
25	337* [258–517]	227–803	216–861	96 [79–112]	63–137	63–145
30	405* [309–620]	272–963	259–1033	115 [94–134]	76–165	75–174

Table compiled by the authors based on their own data

Note: CI — confidence interval; * — statistically significant differences for the median number of cells upon *in vitro* gamma-irradiation at doses of 1 Gy and 2 Gy, $p < 0.0001$.

Table 3. Number of metaphases of T-lymphocytes with dicentric chromosomes (in accordance with the sequence number of analyzed cell)

Sequence number of dicentric chromosomes	Irradiation dose					
	1 Gy			2 Gy		
	Number of T-lymphocyte			Number of T-lymphocyte		
	Median [CI]	10–90 percentiles	min–max	Median [CI]	10–90 percentiles	min–max
1	4* [1–26]	1–25	1–26	2 [2–4]	1–6	1–6
2	28* [8–42]	8–70	8–81	8 [5–10]	2–13	2–14
3	35* [9–72]	10–97	9–107	11 [7–16]	4–28	4–31
4	43* [14–74]	19–112	14–123	14 [10–19]	6–30	5–32
5	56* [27–85]	30–127	27–138	16 [12–25]	6–31	5–32
10	103* [60–115]	62–182	60–192	40 [6–24]	12–70	11–71
15	146* [120–182]	133–425	120–486	49 [44–78]	29–90	24–91
20	177* [157–217]	162–419	157–457	67 [59–94]	41–113	34–116
25	237* [195–289]	195–426	195–426	87 [77–112]	51–125	42–128
30	278* [225–349]	225–405	225–405	105 [96–129]	72–147	70–151

Table compiled by the authors based on their own data

Note: CI — confidence interval; * — statistically significant differences for the median number of T-lymphocytes upon irradiation at doses of 1 Gy and 2 Gy, $p < 0.001$.

higher than the experimentally obtained value, i.e., 0.032 and 0.019 cells, respectively.

Upon *in vitro* irradiation at a dose of 2 Gy, the median expected number of T-lymphocytes with one dicentric chromosome was statistically significantly higher than that obtained from direct slide analysis (4 vs. 2, $p = 0.033$). In all other cases, no statistically significant differences were found.

The analysis showed that identifying the first dicentric chromosomes in *in vitro* gamma-irradiated T-lymphocytes at doses of 1 and 2 Gy requires an analysis of a relatively small number of cells. According to our and literature data, the frequency of dicentric chromosomes in unirradiated samples ranged 0–2 per 1000 analyzed cells [15].

Table 4 shows the probability of detecting the first dicentric chromosome in unirradiated samples. Thus, when analyzing 50 cells, it was 4.9%; when analyzing 100 cells, it was 9.4%. The probability of detecting two dicentric chromosomes in 100 T-lymphocyte metaphases was 2%.

Thus, given the probability of detecting two dicentric chromosomes upon analyzing the first 100 T-lymphocyte metaphases in unirradiated samples, it is necessary to increase the number of identified dicentric chromosomes to indicate radiation exposure. Based on the data presented in Table 3, to identify radiation exposure and classify a sample into a respective dose range, it is proposed to evaluate the number of T-lymphocyte metaphases containing the fifth dicentric chromosome in the order of cytogenetic slide analysis.

For instance, the median number of T-lymphocyte metaphases with the fifth dicentric chromosome was 56 [95% CI: 27; 85] for *in vitro* gamma-irradiation at a dose of 1 Gy and 16 [95% CI: 12; 25] for *in vitro* gamma-irradiation at a dose of 2 Gy ($p < 0.001$). The choice of the median and its corresponding confidence interval as the criterion for dividing exposed individuals into dose ranges is based on its robustness against the influence of outliers, which are characteristic of distributions containing inter-individual differences in radiosensitivity. Although the accuracy of the median estimate is clearly linked to sample size,

increasing the latter will only partially reduce the uncertainty in estimating the central tendency. We believe that the reduction in the confidence interval will be insignificant due to individual responses to radiation. Therefore, the use of median values and the confidence interval appear to be an optimal solution at this stage of the research.

To make a decision regarding the classification of a potentially irradiated sample into one of the dose ranges, it is necessary to analyze the number of cells in which five dicentric chromosomes are identified. Based on the preliminary results, the following algorithm for analyzing T-lymphocyte metaphases is proposed:

1. Determine the sequence number of the analyzed T-lymphocyte metaphase containing the fifth dicentric chromosome in the order of analysis.
2. When the fifth dicentric chromosome in the order of analysis is identified within the first 26 cells, then the irradiated sample should be classified into the radiation exposure dose range of 2 Gy or higher.
3. When the fifth dicentric chromosome is identified between the 27th and 85th T-lymphocyte metaphase during analysis, the irradiated sample should be classified into the radiation exposure dose range of 1 Gy or higher.
4. When fewer than five dicentric chromosomes are identified upon analysis of 85 T-lymphocyte metaphases, the irradiated sample should be classified into the exposure dose range below 1 Gy.
5. When no dicentric chromosomes are detected upon analysis of 50 T-lymphocyte metaphases, there is a 94% probability that the absorbed gamma-irradiation dose of the sample is close to zero.

Table 5 presents the proportion of examined individuals for whom the sequence number of the T-lymphocyte containing the fifth dicentric chromosome falls within the range calculated according to the algorithm described above.

All non-irradiated samples were classified into the “< 1 Gy” group; 100% of them were assigned to the group where the radiation dose is close to zero. At the same time, according to the algorithm, two (16.7%) samples irradiated at a dose of 1 Gy were classified into the “< 1 Gy” group, and two (16.7%) samples irradiated

Table 4. Probability of dicentric chromosome occurrence depending on the number of analyzed cells (in unirradiated samples), %

Number of dicentric chromosomes	Number of analyzed T-lymphocyte metaphases, abs. units				
	10	20	30	50	100
	Probability, %				
0	98.9	97.6	96.4	94.1	88.6
1	0.9	2	3	4.9	9.4
2	0.2	0.4	0.6	1.0	2

Table compiled by the authors based on their own data

Table 5. Distribution of the studied blood samples into dose groups according to the algorithm criteria

Parameter	Dose range			
	≥ 2 Gy	≥ 1 Gy	< 1 Gy	Dose close to 0 Gy
Proportion of individuals, % (abs. units)	83.3 (10)	83.3 (10)	100 (12)	100 (12)
of which:				
False-negative result, % (abs. units)	16.7 (2)	16.7 (2)	–	–

Table compiled by the authors based on their own data

Note: “–” — not identified.

at a dose of 2 Gy were classified into the “≥ 1 Gy or higher” group.

The preliminary results of our study revealed a potential issue of false-negative identification, associated with a possible underestimation of the calculated dose range for a certain proportion of irradiated samples. For medical triage, however, a conservative approach is preferable, where an exposed individual is assigned to a dose group with higher exposure values. A conservative approach reduces the risk of underestimating ionizing radiation exposure and ensures that all potentially exposed individuals receive the necessary medical care. At the next stage of the research, it will be necessary to refine the criteria for assigning an irradiated sample to a particular dose group, taking into account the requirements of conservatism.

According to the literature, the lower limit of detection for the method of scoring unstable chromosomal exchanges depends on the number of analyzed cells and averages 100–250 mGy for sparsely ionizing radiation. At higher radiation doses (5–6 Gy and above), significant impairment of cellular proliferation occurs, resulting in fewer cells reaching metaphase and being included in the cytogenetic analysis [16–18]. Medical triage of exposed individuals based on the frequency of dicentric chromosomes or unstable chromosomal exchanges in T-lymphocytes should be conducted by reducing the number of analyzed T-lymphocyte metaphases to 50 per person, which will significantly increase laboratory throughput. The use of 20–50 cells for analysis increases the confidence intervals for estimating radiation doses assessed using a calibration curve. However, the dose intervals of 0–0.75 Gy and 2–3 Gy are clearly distinguishable when analyzing 50 cells [19, 20], which is consistent with the results obtained in this study.

It is important to note that the present study did not aim to accurately determine accidental exposure doses based on the dose-effect relationship. Under conditions of limited equipment and human resources, it is necessary to develop criteria that, by reducing the

number of analyzed cells, will enable timely assignment of the exposed population into groups at risk of developing the hematopoietic form of ARS. This will facilitate the planning and adjustment of further medical follow-up. Following cytogenetic triage, it is possible to increase the number of analyzed cells for biodosimetry purposes.

In future studies, we aim to validate the developed method on other sample sets, conduct inter-laboratory comparisons, and, based on the data obtained, optimize the algorithm for assessing dose intervals.

CONCLUSION

One of the tasks in providing emergency medical care to exposed individuals during mass radiation incidents is to conduct medical triage aimed at identifying persons at high risk of developing ARS. In this work, we have developed a preliminary cytogenetic triage algorithm, based on determining the number of analyzed T-lymphocyte metaphases containing the fifth dicentric chromosome in the order of their analysis.

It was established that when the fifth dicentric chromosome is identified within the first 26 analyzed T-lymphocyte metaphases, the radiation dose falls within the range of 2 Gy or higher. When the fifth dicentric chromosome is identified between the 27th and 85th analyzed T-lymphocyte metaphase, the radiation dose falls within the range of 1 Gy or higher. In the case of analyzing 85 T-lymphocyte metaphases with fewer than 5 dicentric chromosomes identified, the radiation dose falls within the range below 1 Gy. In cases where no dicentric chromosomes are detected upon analysis of 50 T-lymphocyte metaphases, the radiation dose falls within the range below 1 Gy and/or is close to zero values. This algorithm is preliminary and requires further refinement, taking into account a conservative approach to reduce the risk of underestimating ionizing radiation exposure. Continuation of the research is planned to refine the algorithm and validate the results obtained.

References

1. Kutkov VA. Substantive provisions of the IAEA guidelines for criteria for protecting the public and workers in radiation emergency. *Radiation and Risk*. 2006;15(3–4):133–56 (In Russ.). EDN: [JWSDRF](#)
2. Guskova AK, Krasnyuk VI, Galstyan IA, Nadejina NM. 30 years of Chernobyl NPP accident: experience of mitigation of medical consequences. *Medical Radiology and Radiation Safety*. 2016;61(3):30–5 (In Russ.). EDN: [VWDLJD](#)
3. Samoylov AS, Konchalovsky MV, Bushmanov AYU, Galstyan IA, Nugis VYu, Davtyan AA, et al. Recommendations for the diagnosis and treatment of bone marrow form of acute radiation syndrome. *Russian Journal of Hematology and Transfusiology*. 2023;68(1):98–128 (In Russ.). <https://doi.org/10.35754/0234-5730-2023-68-1-98-128>
4. Bushmanov AYU, Galstyan IA, Kashirina OG, Konchalovsky MV, Lisunov VYu, Metlyaeva NA, et al. Results of long-term medical observation of direct participants of the accident at the Chernobyl NPP in the Russian Federation. *A.I. Burnasyan Federal Medical Biophysical Center Clinical Bulletin*. 2024;4:51–9 (In Russ.). <https://doi.org/10.33266/2782-6430-2024-4-51-59>
5. Sedankin MK, Gudkov EA, Soloviev VYu, Mershin LYU. Features of using a lymphocyte test for biological dosimetry in the early period after exposure. *Extreme Medicine*. 2023;25(3):65–70 (In Russ.). <https://doi.org/10.47183/mes.2023.034>
6. Sullivan JM, Prasanna PG, Grace MB, Wathen LK, Wallace RL, Koerner JF, et al. Assessment of biodosimetry methods for a mass-casualty radiological incident: medical response and management considerations. *Health Physics*. 2013;105(6):540–54. <https://doi.org/10.1097/hp.0b013e31829cf221>
7. Coleman CN, Sullivan JM, Bader JL, Murrain-Hill P, Koerner JF, Garrett AL, et al. Public health and medical preparedness for a nuclear detonation: the nuclear incident medical enterprise. *Health Physics*. 2015;108(2):149–60. <https://doi.org/10.1097/hp.0000000000000249>
8. International Atomic Energy Agency. *Cytogenetic analysis for radiation dose assessment: a manual*. Vienna: IAEA; 2001.
9. Neronova EG. Calibration curves development for biological cytogenetic dosimetry and experience with medical exposure. *Medico-Biological and Socio-Psychological Problems of Safety in Emergency Situations*. 2021;4:85–93 (In Russ.). EDN: [JAWXRM](#)
10. Kuznetsova TV, Shilova NV, Tvorogova MG, Kharchenko TV, Lebedev IN, Antonenko VG. Practical recommendations to ensure quality and safety of cytogenetic research. *Medical Genetics*. 2019;18(5):3–27 (In Russ.). <https://doi.org/10.25557/2073-7998.2019.05.3-27>
11. Port M, Barquinero JF, Endesfelder D, Moquet J, Oestreich U, Terzoudi G, et al. RENE Inter-Laboratory Comparison 2021: Inter-Assay Comparison of Eight Dosimetry Assays. *Radiation Research*. 2023;199(6):535–55. <https://doi.org/10.1667/rade-22-00207.1>
12. Pinto MM, Santos NF, Amaral A. Current status of biodosimetry based on standard cytogenetic methods. *Radiation and Environmental Biophysics*. 2010;49(4):567–81. <https://doi.org/10.1007/s00411-010-0311-3>
13. Vijayalakshmi J, Chaurasia RK, Srinivas KS, Vijayalakshmi K, Paul SFD, Bhat NN, et al. Establishment of ex vivo calibration curve for X-ray induced “dicentric + ring” and micronuclei in human peripheral lymphocytes for biodosimetry during radiological emergencies, and validation with dose blinded samples. *Heliyon*. 2023;9(6):e17068. <https://doi.org/10.1016/j.heliyon.2023.e17068>
14. Vozilova AV, Akhmadullina YR. Study of the individual radiosensitivity in humans based on the assessment of the frequency of chromosome aberrations and micronuclei in peripheral blood T lymphocytes. *Russian Journal of Genetics*. 2019;55:1234–41. <https://doi.org/10.1134/S1022795419100156>
15. Ainsbury E, Barquinero JF, Beinke C, Blakely WF, Braselmann H, Carr Z, et al. *Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies*. Vienna: IAEA; 2011. EDN: [YPPDFP](#)
16. Bauchinger M. *Chromosome painting and biological dosimetry of absorbed radiation*. Proceedings of the Tenth International Congress of Radiation Research. Würzburg; 1995.
17. Flegal FN, Devantier Y, Marro L, Wilkins RC. Validation of QuickScan dicentric chromosome analysis for high throughput radiation biological dosimetry. *Health Physics*. 2012;102:143–153. <https://doi.org/10.1097/hp.0b013e3182307758>
18. Testa A, Palma V, Patrono C. Dicentric Chromosome Assay (DCA) and Cytokinesis-Block Micronucleus (CBMN) Assay in the Field of Biological Dosimetry. In: Dhawan A, Bajpayee M (eds). *Genotoxicity Assessment. Methods in Molecular Biology*, vol 2031. New York: Humana; 2019:105–19. https://doi.org/10.1007/978-1-4939-9646-9_5
19. Romm H, Wilkins RC, Coleman CN, Lillis-Hearne PK, Pellmar TC, Livingston GK, et al. Biological dosimetry by the triage dicentric chromosome assay: potential implications for treatment of acute radiation syndrome in radiological mass casualties. *Radiation Research*. 2011;175(3):397–404. <https://doi.org/10.1667/RR2321.1>
20. Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC. The role of cytogenetics in early triage of radiation casualties. *Applied Radiation and Isotopes*. 2000;52(5):1107–12. [https://doi.org/10.1016/S0969-8043\(00\)00054-3](https://doi.org/10.1016/S0969-8043(00)00054-3)

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EARLY AND LONG-TERM ALTERATIONS IN SENSORIMOTOR PARAMETERS FOLLOWING FRACTIONATED GAMMA IRRADIATION IN AN EXPERIMENTAL SETTING

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Introduction. Epidemiological studies suggest that ionizing radiation increases the risk of developing neurodegenerative diseases long after exposure; however, there is a notable lack of longitudinal studies and experimental research into establishing causal relationships between radiation dose and potential neurodegenerative effects.

Objective. The work investigates early and long-term alterations in sensorimotor parameters characterizing coordination in laboratory animals subjected to a range of doses of fractionated gamma irradiation at a young age.

Materials and methods. Experiments were carried out on mice of both sexes ($n = 400$) of the C57Bl/6 strain. Five observation groups were formed: two control groups and three groups with different radiation exposure levels, each made up of 80 individuals (40 males and 40 females). The animals underwent total external gamma irradiation during their first month of life at cumulative doses of 0.1 Gy, 1 Gy, and 5 Gy, with each dose divided into 20 fractions. The control groups of non-irradiated mice included: a group of intact animals serving as biological control ($n = 80$) and a sham-irradiation placebo group ($n = 80$). Evaluation of motor function is an effective tool for assessing symptoms of neurodegenerative diseases in animals. Coordination in irradiated and control animals was assessed using the tapered beam walking test at the ages of 1, 6, 12, and 18 months. Data were analyzed using Microsoft Excel and the R programming language.

Results. Sex and age were shown to influence sensorimotor parameters characterizing motor coordination (better in females, worsens with age in animals of both sexes), while no significant effect of the stress factor associated with animal irradiation was found on the studied parameters. A comparison of sex- and age-standardized sensorimotor parameters between irradiated animals and non-irradiated control mice revealed dose-dependent alterations. A deteriorated in motor coordination in the long term in mice exposed to gamma irradiation at a cumulative dose of 5 Gy manifested itself by a more than 1.5-fold increase in the number of errors compared to non-irradiated animals ($t = 6.7$; $p < 0.001$). Conversely, irradiation at a cumulative dose of 0.1 Gy produced an opposite effect: both in the early and long-term periods, the speed of mouse movement along the tapered beam increased relative to the non-irradiated control (walking time decreased by an average of 20% ($p < 0.001$) at any age), while in the early period, the number of errors also decreased ($t = 2.36$; $p = 0.02$), indicating an improvement in coordination ability.

Conclusions. Fractionated gamma irradiation at a young age induced dose-dependent alterations in sensorimotor function in animals: irradiation at a cumulative dose of 0.1 Gy resulted in improved coordination both in the early and long-term periods post-exposure; whereas irradiation at a cumulative dose of 5 Gy led to signs of impaired motor coordination in the long term, at 18 months of age.

Keywords: irradiation, motor coordination, sensorimotor function, neurodegenerative diseases, long-term effects

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РАННИЕ И ОТДАЛЕННЫЕ ИЗМЕНЕНИЯ СЕНСОМОТОРНЫХ ПОКАЗАТЕЛЕЙ ПРИ ФРАКЦИОНИРОВАННОМ ГАММА-ОБЛУЧЕНИИ В ЭКСПЕРИМЕНТЕ

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Введение. Эпидемиологические исследования позволяют предположить, что ионизирующее излучение повышает риск развития нейродегенеративных заболеваний в отдаленном периоде после облучения, однако отмечается нехватка лонгитюдных исследований и экспериментальных работ для установления причинно-следственных связей между дозой облучения и потенциальными нейродегенеративными эффектами.

Цель. Изучение ранних и отдаленных изменений сенсомоторных показателей, характеризующих координацию у лабораторных животных, подвергнутых в раннем возрасте фракционированному гамма-излучению в различных дозах.

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Материалы и методы. Эксперимент выполнен на мышах обоих полов ($n = 400$) линии C57Bl/6. Формировали 5 групп наблюдения: 2 контрольные группы и 3 группы с различными уровнями облучения по 80 особей каждая (40 самцов и 40 самок). Животных подвергали общему внешнему гамма-облучению в течение первого месяца жизни в кумулятивных дозах 0,1, 1 и 5 Гр, каждая доза была разделена на 20 фракций. Контрольные группы необлученных мышей включали: группу интактных животных — биологический контроль ($n = 80$) и группу ложного облучения ($n = 80$). Эффективный инструмент оценки симптомов нейродегенеративных заболеваний у животных — оценка моторной функции. Оценивали координацию облученных и контрольных животных в тесте ходьбы по сужающейся перекладине в возрасте 1, 6, 12 и 18 месяцев. Данные анализировали в программе Microsoft Excel и с использованием языка программирования R.

Результаты. Выявлено влияние пола и возраста на сенсомоторные показатели, характеризующие координацию движений (лучше у самок, ухудшается с возрастом у животных обоих полов), при этом не обнаружено существенного влияния на изучаемые параметры фактора стресса, связанного с облучением животных. При сравнении стандартизованных по полу и возрасту сенсомоторных показателей у облученных животных и контрольных необлученных мышей выявлены зависимые от дозы изменения. Координация движений ухудшалась в отдаленные сроки у мышей при гамма-облучении в кумулятивной дозе 5 Гр, это проявлялось в увеличении числа ошибок более чем в 1,5 раза по сравнению с необлученными животными ($t = 6,7$; $p < 0,001$). При облучении в кумулятивной дозе 0,1 Гр обнаружен обратный эффект: как в раннем, так и в отдаленном периоде повышалась скорость перемещения мышей по сужающейся перекладине относительно необлученного контроля (время ходьбы снижалось в среднем на 20% ($p < 0,001$) в любом возрасте), в раннем периоде также снижалось число ошибок ($t = 2,36$; $p = 0,02$), что указывает на улучшение способности к координации.

Выводы. Фракционированное гамма-облучение в раннем возрасте у животных вызвало зависимые от дозы изменения сенсомоторной функции: при облучении в кумулятивной дозе 0,1 Гр и в раннем, и в отдаленном периоде после облучения отмечено улучшение координации; при облучении в кумулятивной дозе 5 Гр в отдаленном периоде, в возрасте 18 мес., выявлены признаки ухудшения координации движений у мышей.

Ключевые слова: облучение; координация движений; сенсомоторная функция; нейродегенеративные заболевания; отдаленные эффекты

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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 Уральского научно-практического центра радиационной медицины² ФМБА (протокол № 1 от 03.04.2023).

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INTRODUCTION

Increased interest in radiation neurobiology is largely due to research in space medicine and the need to ensure protection against space radiation during future manned interplanetary flights. The salient characteristics of space radiation, comprised of Earth's radiation belts (protons and electrons), solar cosmic rays (protons), and galactic cosmic rays (accelerated hydrogen and helium ions, heavy charged particles), lead to significant non-uniformity of the radiation dose both in space and time due to the unpredictability of solar proton events. Therefore, research into the effects of chronic and protracted fractionated irradiation, including the study of mechanisms underlying central nervous system impairments, is of particular importance for space radiobiology [1]. In addition to the use of radiation therapy for cancer patients and the widespread application of medical diagnostic procedures involving radiation exposure, a comprehensive analysis of the impact of protracted,

chronic, or fractionated irradiation on the brain is driven by the need to protect the health of nuclear industry professionals.

For a number of radiation-induced effects on the central nervous system, a causal relationship with radiation exposure has been established including the determination of threshold values for absorbed dose. For instance, acute exposure exceeding 1–2 Gy in adults or 0.1 Gy in young children may increase the risk of cognitive dysfunction (International Commission on Radiological Protection Publication 118³). Cognitive dysfunction in rodents, which is manifested as impaired memory or reduced learning ability in humans, along with impaired spatial learning, novel object recognition, and decision-making function, is associated with radiation effects, primarily in the hippocampal region [2]. The mechanisms underlying these impairments involve radiation-induced oxidative stress, which disrupts normal proliferation and differentiation of neuronal stem cells, impairs synaptic plasticity, triggers the activation of

² Currently, it is the Southern Urals Federal Research and Clinical Center for Medical Biophysics.

³ ICRP Statement on tissue reactions. Early and late effects of radiation in normal tissues and organs — threshold doses for tissue reactions in a radiation protection context. ICRP Publication 118. Ann. ICRP 41(1/2); 2012.

pro-inflammatory cytokines, and, at high doses, leads to neuronal death [2].

Regarding other consequences of ionizing radiation exposure on the brain, there is less epidemiological and experimental data. For instance, several studies discuss the potential increased risk of developing neurodegenerative diseases long after radiation exposure [3]. In a cohort of Russian nuclear industry workers chronically exposed to radiation during their professional activities at a nuclear fuel production facility (Mayak Production Association, Ozersk, Chelyabinsk Region), an analysis of the dose-response relationship, accounting for non-radiation factors (sex, age), revealed an association between the incidence of Parkinson's disease and the cumulative dose from external gamma radiation [4].

In a study of causes of death in a French cohort of nuclear industry workers (SELTINE, 1968–2014), statistically significant dose-risk relationships were established for increased mortality from leukemia and dementia, although the authors noted that the observed association between the risk of death from dementia and low-dose radiation should be interpreted with caution at this stage [5]. However, for scenarios of acute exposure in the dose range of 0–4 Gy (Japanese cohort of atomic bomb survivors in Hiroshima and Nagasaki), studies did not find an increased incidence of dementia [6] or a correlation between age-related cognitive decline and radiation dose [7].

In a study carried out on patients with multiple sclerosis in the period prior to the onset of symptoms related to this disease and confirmation of the diagnosis, Motamed et al. found that this cohort had been more frequently exposed to diagnostic or therapeutic X-ray irradiation and had a higher total accumulated dose than individuals in the control group without multiple sclerosis; based on this, the authors concluded a possible association between the dose of X-ray irradiation and the risk of developing this disease [8].

In a study investigating the risks of developing non-cancer central nervous system diseases following exposure in adulthood, Lopes et al. revealed a significant positive relative risk for the development of cerebrovascular diseases and Parkinson's disease; however, the authors noted several methodological issues, such as difficulties in reconstructing individual radiation doses and limited accounting for potential confounding factors [9]. Particular challenges when studying the mechanisms of ionizing radiation's influence on neurodegenerative and age-related changes arise as a result of the long latency period from exposure to the potential development of disease signs.

Currently, there is a lack of longitudinal studies to establish causal relationships between radiation dose and potential risks of developing neurodegenerative diseases [2, 3].

At the same time, no attempts have been made to study the association between the development of neurodegenerative diseases long after irradiation in

experimental animals [3]. On the one hand, radiation-induced pro-inflammatory microglial activation and oxidative stress increase the risk of neurodegenerative processes; on the other hand, certain irradiation regimens have demonstrated a neuroprotective effect in experiments carried out on a transgenic animal model of Alzheimer's disease [10].

One of the simplest and most effective methods for the experimental assessment of neurodegenerative disease symptoms using laboratory animals involves investigations into motor function [11]. Sensorimotor effects measured in animals following experimental exposures indirectly indicate alterations in other behavioral parameters, such as cognitive functions and emotions [12]. A number of behavioral tests developed to assess sensorimotor functions in rodents enable the evaluation of sensorimotor information processing speed, locomotor function, balance, grip strength, motor coordination, orienting-exploratory response, etc. [12].

The present study investigates early and long-term alterations in sensorimotor parameters characterizing coordination in laboratory animals subjected to fractionated gamma irradiation at a young age across various doses. The work, which is a continuation of previously published research into early alterations in sensorimotor parameters in fractionally irradiated mice [13], aims to supplement the previously obtained results with information on the first identified impairment of coordination in irradiated animals in the long term after exposure.

MATERIALS AND METHODS

The experiment was performed on mice of both sexes ($n = 400$) of the C57Bl/6 strain (Specific Pathogen Free (SPF), vivarium of the Institute of Cytology and Genetics, SB RAS, Novosibirsk). The animals were subjected to fractionated gamma-irradiation from birth until 30 days. Subsequent assessments of sensorimotor parameters were conducted in the irradiated animals four times over a period of 18 months. Five observation groups were formed: two control groups and three groups with different radiation levels, each group being comprised of 80 individuals each (40 males and 40 females). With age, the number of examined animals gradually decreased due to the natural death of some of them.

The exposed animal groups included the "5 Gy" group ($n = 80$), the "1 Gy" group ($n = 80$), and the "0.1 Gy" group ($n = 80$). Mice in these groups were exposed to cumulative doses of 5 Gy, 1 Gy, and 0.1 Gy, respectively. In each case, the cumulative dose was divided into 20 equal fractions of 0.25 Gy, 0.05 Gy, and 0.005 Gy, respectively. Irradiation was performed over four weeks, five days per week, using the experimental radiobiological setup IGR-1M (Kvant, Russia), equipped with ^{137}Cs sources. For exposure to single doses of 0.25 Gy and 0.05 Gy, the dose rate was 0.72 Gy/min. To achieve a single dose of 0.005 Gy, lead collimators were used (the dose rate when using collimators was 10 mGy/min).

The non-uniformity of the gamma field within the working space did not exceed 10%. Mice were exposed in their permanent housing cages; lactating females were temporarily removed during the exposure procedure.

The control groups of non-exposed mice included: a group of intact animals comprising the biological control (BC) ($n = 80$) and a sham-exposed placebo group (0 Gy) ($n = 80$). The animals in the “0 Gy” group underwent the same manipulations as those in the exposed groups (with the exception of the radiation exposure itself) to account for the influence of stress factors associated with animal handling and temporary maternal separation on the studied parameters.

Following the completion of the exposure, the animals at an age of 28–30 days were transferred to separate cages (10 males and 10 females per cage) for their permanent accommodation in a conventional vivarium (at a temperature of $22 \pm 2^\circ\text{C}$, with unlimited access to drinking water and complete granulated feed).

To assess sensorimotor function, the animals were tested at the ages of 1, 6, 12, and 18 months.

The raised-beam walking test, designed to assess motor deficits primarily in the hind limbs, can reveal pathologies of the motor cortex and evaluate coordination in rodents [14, 15]. The test evaluated the ability of an individual animal to traverse a 1-meter-long, elevated, tapering, two-level beam. Over two days, the mice were trained to traverse the beam to reach a safe shelter. On the third day, the traversal of the setup by the mice was recorded on video (Sony $\alpha 37$ camera).

The following parameters were recorded: total time taken to traverse the beam from the start point to the shelter, foot slips off the beam surface, falls, and the number of limb placements on the lower ledge (errors) [14, 15]. Parameters were recorded using the RealTimer software (Open Science RPC, Russia). If an animal fell off the setup or failed to reach the shelter during testing on the 3rd day, it was excluded from further analysis (in each experimental group, the number of such animals did not exceed 1–2 individuals).

The results were expressed as mean values and standard errors ($M \pm SE$). Since all analyzed parameters conformed to a normal distribution according to the Kolmogorov–Smirnov test, comparison of mean values between experimental and control groups was performed using parametric methods with Student's t -test. Results were considered statistically significant at $\alpha = 0.05$.

Pearson's correlation analysis was performed to assess the association of the studied parameters characterizing sensorimotor function in the animals. Regression analysis was used to describe the influence of age on the investigated parameters. Multifactorial analysis of variance (ANOVA) in the general linear model was used to identify the effects of radiation dose and other concomitant factors (sex, age, radiation-associated stress). When assessing the stress factor, it was assumed that

only the intact animals in the BC group did not experience stress associated with the early-life exposure procedure. Data were analyzed using Microsoft Excel and the R programming language.⁴ Pairwise comparisons of parameter values across different dose groups were performed to establish the significance of differences between groups exposed to different doses. For this purpose, data were normalized for age and sex, followed by *post-hoc* analysis in a one-way ANOVA with Bonferroni correction.

RESULTS AND DISCUSSION

During the raised-beam walking test, the time taken by the animal to traverse a 1-m distance on an elevated, tapering beam was recorded, along with events such as limb placements on the lower ledge of the setup (errors), limb slips off the beam, and pauses.

Table 1 presents the measured parameters of the raised-beam walking test for mice in the different experimental groups. Generally, a lower number of errors, pauses, and/or a shorter beam traversal time was observed in females compared to males within the same group. For instance, statistically significant differences between males and females were noted at the age of 1 month in the “0 Gy” and “1 Gy” groups for the parameter of total walking time, as well as in the “0.1 Gy” group for the number of errors.

At 6 months of age, females exhibited a statistically significant reduction compared to males in the number of pauses in the BC group, the number of errors in the “0.1 Gy” group, and the walking time in the “1 Gy” group. At 12 months, a statistically significant reduction in total walking time in females was observed in the BC, “0.1 Gy”, and “1 Gy” groups; a reduction in the number of pauses was seen in the “0.1 Gy” and “1 Gy” groups; a reduction in the number of slips occurred in the BC and “5 Gy” groups; and a reduction in the number of errors was found in the “0 Gy” and “0.1 Gy” groups. At 18 months, a reduction in total walking time was noted in the BC, “0 Gy”, and “1 Gy” groups, while a reduction in the number of pauses was observed in the BC, “0 Gy”, and “0.1 Gy” groups (Table 1).

From the comparison of sensorimotor function parameters in non-exposed mice from the “0 Gy” and BC groups, no definitive conclusions can be drawn regarding the influence of the stress factor on these parameters. For instance, at 1 month, females in the “0 Gy” group made fewer errors than females in the BC group, whereas at 6 months, the number of errors in “0 Gy” females became statistically significantly higher than in the BC group. At 12 months, animals of both sexes in the “0 Gy” group had a shorter total walking time compared to the BC group; additionally, males made fewer slips and errors relative to the BC group, which could be interpreted as improved coordination. At 18 months, males in the “0 Gy” group had statistically significantly

⁴ R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2022. <https://www.R-project.org>

Table 1. Raised-beam walking test parameters in mice across different experimental groups

Age, months	Group	Sex	Errors	Slips	Total walking time, s	Pauses
1	BC	♂ (<i>n</i> = 40)	8 ± 0.50	0.8 ± 0.18	5.6 ± 0.28	3.2 ± 0.51
		♀ (<i>n</i> = 40)	8.5 ± 0.77	0.7 ± 0.24	5.4 ± 0.21	2.8 ± 0.34
	0 Gy	♂ (<i>n</i> = 37)	7.2 ± 0.83	0.7 ± 0.22	5.7 ± 0.31	3.2 ± 0.38
		♀ (<i>n</i> = 37)	6.5 ± 0.51*	0.6 ± 0.21	4.8 ± 0.24 [□]	2.6 ± 0.38
	0.1 Gy	♂ (<i>n</i> = 40)	7.6 ± 0.78	0.7 ± 0.19	4.2 ± 0.13**	2 ± 0.37*
		♀ (<i>n</i> = 40)	4.8 ± 0.42** [□]	0.6 ± 0.24	4.2 ± 0.22*	1.8 ± 0.24*
	1 Gy	♂ (<i>n</i> = 40)	8 ± 0.89	0.7 ± 0.22	5 ± 0.24	2.8 ± 0.40
		♀ (<i>n</i> = 41)	6.6 ± 0.64	0.7 ± 0.18	4.3 ± 0.16* [□]	1.9 ± 0.24*
	5 Gy	♂ (<i>n</i> = 40)	9.1 ± 0.86	0.8 ± 0.17	5.4 ± 0.28	2.5 ± 0.30
		♀ (<i>n</i> = 39)	7.4 ± 0.65	0.8 ± 0.23	5 ± 0.22	2 ± 0.25
6	BC	♂ (<i>n</i> = 40)	7.6 ± 0.55	0.9 ± 0.15	6.2 ± 0.21	3 ± 0.28
		♀ (<i>n</i> = 40)	6.9 ± 0.62	0.5 ± 0.10	6 ± 0.24	2.1 ± 0.24 [□]
	0 Gy	♂ (<i>n</i> = 40)	9.8 ± 1.01	0.7 ± 0.13	6.4 ± 0.35	3.5 ± 0.44
		♀ (<i>n</i> = 40)	9.6 ± 0.78*	0.5 ± 0.09	5.8 ± 0.25	2.8 ± 0.32
	0.1 Gy	♂ (<i>n</i> = 40)	10.3 ± 0.77*	0.7 ± 0.13	5.1 ± 0.21**	2.3 ± 0.20*
		♀ (<i>n</i> = 39)	7.7 ± 0.48* [□]	0.4 ± 0.08	4.7 ± 0.25**	1.9 ± 0.23*
	1 Gy	♂ (<i>n</i> = 40)	10.6 ± 0.76*	1 ± 0.15	5.8 ± 0.24	2.8 ± 0.33
		♀ (<i>n</i> = 40)	10.3 ± 0.76*	0.9 ± 0.15*	5 ± 0.17** [□]	2.7 ± 0.34
	5 Gy	♂ (<i>n</i> = 40)	8.8 ± 0.57	0.8 ± 0.13	5.4 ± 0.24**	2.6 ± 0.32
		♀ (<i>n</i> = 39)	8.8 ± 0.76	0.7 ± 0.12	4.8 ± 0.20**	2.4 ± 0.30
12	BC	♂ (<i>n</i> = 40)	9.8 ± 0.73	1.3 ± 0.17	6.8 ± 0.26	3.2 ± 0.44
		♀ (<i>n</i> = 40)	8 ± 0.75	0.6 ± 0.13 [□]	6 ± 0.24 [□]	2.6 ± 0.28
	0 Gy	♂ (<i>n</i> = 40)	7.1 ± 0.68*	0.6 ± 0.11*	5.4 ± 0.22*	3 ± 0.31
		♀ (<i>n</i> = 40)	9.9 ± 0.74 [□]	0.4 ± 0.11	4.7 ± 0.25*	2.3 ± 0.35
	0.1 Gy	♂ (<i>n</i> = 40)	9.9 ± 0.77*	0.5 ± 0.11*	5.1 ± 0.19*	2.4 ± 0.27
		♀ (<i>n</i> = 38)	7.6 ± 0.66* [□]	0.4 ± 0.09	4.6 ± 0.14* [□]	1.5 ± 0.23* [□]
	1 Gy	♂ (<i>n</i> = 40)	8.8 ± 0.87	0.4 ± 0.10*	6.1 ± 0.25*	2.9 ± 0.29
		♀ (<i>n</i> = 40)	9.8 ± 0.65	0.4 ± 0.09	5.2 ± 0.19* [□]	1.9 ± 0.25 [□]
	5 Gy	♂ (<i>n</i> = 37)	11.3 ± 0.83*	0.8 ± 0.14*	6.3 ± 0.38*	2.8 ± 0.40
		♀ (<i>n</i> = 34)	9.9 ± 0.78	0.4 ± 0.10 [□]	6.3 ± 0.28*	2 ± 0.32
18	BC	♂ (<i>n</i> = 24)	8.8 ± 0.70	0.2 ± 0.08	7.7 ± 0.39	4.9 ± 0.59
		♀ (<i>n</i> = 32)	9.3 ± 0.75	0.3 ± 0.09	6.4 ± 0.23 [□]	3 ± 0.37 [□]
	0 Gy	♂ (<i>n</i> = 38)	6 ± 0.79*	0.4 ± 0.10	9.5 ± 0.52*	4.7 ± 0.46
		♀ (<i>n</i> = 32)	8.1 ± 1.01	0.4 ± 0.12	6.3 ± 0.36 [□]	2.9 ± 0.40 [□]

Table 1 (continued)

Age, months	Group	Sex	Errors	Slips	Total walking time, s	Pauses
18	0.1 Gy	♂ (<i>n</i> = 29)	8.6 ± 0.81*	0.6 ± 0.15*	5.8 ± 0.34**	2.9 ± 0.44**
		♀ (<i>n</i> = 26)	7.5 ± 0.86	0.3 ± 0.12	5.1 ± 0.21**	1.7 ± 0.27**□
	1 Gy	♂ (<i>n</i> = 38)	10.2 ± 0.63*	0.4 ± 0.11	7 ± 0.33*	3.3 ± 0.26**
		♀ (<i>n</i> = 39)	10 ± 0.65	0.7 ± 0.15*	5.8 ± 0.27□	2.6 ± 0.36
	5 Gy	♂ (<i>n</i> = 34)	13.2 ± 0.76**	0.8 ± 0.13*	7.2 ± 0.34*	3.9 ± 0.43
		♀ (<i>n</i> = 26)	11.9 ± 0.89**	0.5 ± 0.10	6.5 ± 0.30	3.4 ± 0.38

Table compiled by the authors based on original data

Note: BC — biological control; * — statistically significant differences compared to the BC group of the corresponding age, *p* < 0.05; ** — statistically significant differences compared to the “0 Gy” group of the corresponding age, *p* < 0.05; □ — statistically significant differences between females and males within the same group, *p* < 0.05.

Table 2. Results of the multifactorial analysis of variance

Factors	Sensorimotor parameters in the raised-beam walking test							
	Total walking time		Errors		Slips		Pauses	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Age	71.4	< 0.001	15.1	< 0.001	7.6	0.001	13.0	< 0.001
Dose	27.7	< 0.001	12.2	< 0.001	3.7	0.012	13.0	< 0.001
Stress	2.61	0.11	0.72	0.40	7.5	0.006	0.22	0.64
Sex	74.2	< 0.001	6.1	0.014	16.5	< 0.001	43.8	< 0.001

Table compiled by the authors based on original data

Note: *p* — level of statistical significance of differences.

fewer errors than the BC group, but a longer total walking time (Table 1).

Age-related coordination impairment is one of the signs of altered sensorimotor functions accompanying neurodegenerative diseases. A decline in coordination observed as the animals aged was manifested by an increase in beam traversal time and an increased number of pauses. In non-exposed animals (BC and “0 Gy” groups), regression analysis indicated that the relationship between age and the analyzed parameters could be best described by a linear function. A statistically significant effect of age on total walking time ($R^2 = 0.06$; $F = 29.5$; $p < 0.001$) was manifested by an average increase in walking time of 0.112 ± 0.021 seconds per month. A statistically significant positive linear relationship was also found between age and the number of pauses during beam traversal ($R^2 = 0.03$; $F = 12.6$; $p < 0.001$), along with a negative relationship between age and the number of slips ($R^2 = 0.02$; $F = 7.6$; $p = 0.006$). Regression analysis did not reveal a statistically significant relationship between age and the number of errors ($F = 1.3$; $p = 0.26$).

Multifactorial analysis of variance using the general linear model to assess the influence of the studied

factors on sensorimotor function parameters showed that factors such as age, radiation dose, and sex had a statistically significant influence on all investigated sensorimotor parameters (Table 2). In contrast, a statistically significant influence of the stress factor was noted only for the number of limb slips ($F = 7.5$; $p = 0.006$). Based on the results of the multifactorial ANOVA, the time required to traverse the beam increased with age and radiation dose more markedly in males than in females. However, the dependence on radiation dose was non-linear: the minimum traversal times were characteristic of animals exposed to a dose of 0.1 Gy, while in those exposed to 5 Gy, this parameter reached values comparable to the non-exposed control. Similar patterns were observed for the number of pauses during the test. The number of errors during beam traversal increased linearly with both the age of the mice and the radiation dose, being maximal at a dose of 5 Gy and more pronounced in males than in females. The number of slips decreased with age but increased with radiation dose to a greater extent in males than in females.

To assess the effect of radiation exposure on sensorimotor function parameters in mice, the previously identified sex and age differences were taken into account. For

this purpose, the studied parameters were standardized by sex and age: after setting the values for non-exposed animals to 1, the measurements for exposed males and females were normalized to the mean value of the corresponding parameter for males or females in the control group of the same age. Furthermore, since the multi-factorial analysis did not reveal a substantial influence of the stress factor on the studied parameters despite comparisons between the “0 Gy” and “BC” groups using Student’s *t*-test showing non-systematic differences across ages, the exposed animals were compared to a combined control group (BC + “0 Gy”) to describe the changes in their sensorimotor parameters.

The dependence of the investigated parameters on the dose of fractionated radiation for animals of different ages is presented in Figures 1–4.

The study demonstrates the effect of fractionated gamma radiation dose on sensorimotor function. However, the dependence of the investigated parameters on the dose was non-linear. A long-lasting effect (persisting at least until 18 months of age in mice) of improved sensorimotor parameters (coordination) was identified in animals irradiated with a dose of 0.1 Gy compared to non-exposed animals. Specifically, at any age, the time required to traverse the beam and the number of pauses in the “0.1 Gy” group were statistically significantly lower than in the non-exposed animals (Figs. 1, 2). The total walking time decreased by 15–30%, and the number of pauses decreased by 25–40%. At the age of 1 month, the number of errors in this group was also statistically significantly reduced (Fig. 3), measuring 7.6 ± 0.33 in the control group versus 6.2 ± 0.50 in the “0.1 Gy” group ($t = 2.28$; $p = 0.023$).

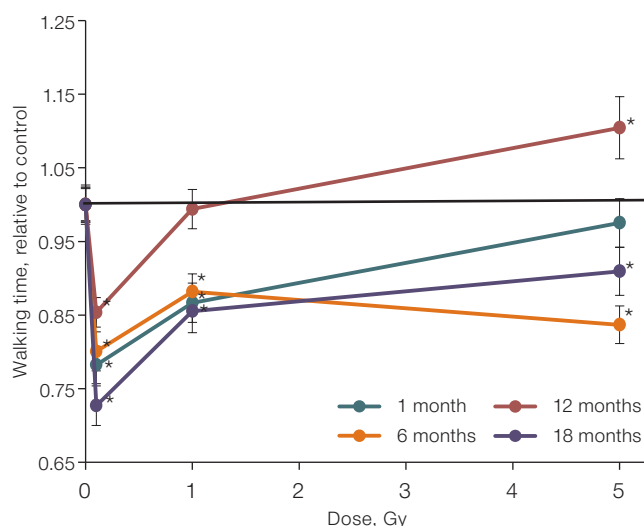


Figure prepared by the authors based on original data

Fig. 1. Change in raised-beam walking time in mice exposed to fractionated gamma radiation at different doses during early development: * — level of statistical significance of differences compared to the non-exposed control group

At 12 months of age, a reduction in the number of slips (Fig. 4) to 0.4 ± 0.11 was observed, compared to 0.70 ± 0.071 in the control group ($t = 2.46$; $p = 0.01$). The decrease in these parameters can be interpreted as a long-term improvement in coordination in animals exposed to a dose of 0.1 Gy.

In the “1 Gy” group, the walking time was statistically significantly shorter by 10–15% as compared to the non-exposed control at the ages of 1, 6, and 18 months (Fig. 1), the number of pauses was lower by 20% at 1 and 18 months (Fig. 2), while the number of slips was reduced by 10% at 1 month and by 40% at 12 months (Fig. 4). On the other hand, the number of errors made by animals in the “1 Gy” group during the test exceeded the control values at 6 months: 10.4 ± 0.52 errors compared to 8.5 ± 0.39 errors in the control group ($t = 3.03$; $p = 0.003$). At 18 months: 10.1 ± 0.54 errors compared to 7.9 ± 0.43 errors in the control group ($t = 3.36$; $p < 0.001$). The corresponding data are presented in Figure 3.

When the radiation dose was increased to 5 Gy, the total walking time and the number of errors and slips increased; however, they did not exceed control values until the age of 12 months. In animals irradiated with a dose of 5 Gy, the beam traversal time was statistically significantly reduced compared to the control at 6 and 18 months (Fig. 1), while at 12 months, an increase in the mean walking time to 6.3 ± 0.2 s was observed compared to 5.7 ± 0.1 s in the control group ($t = 2.46$; $p = 0.014$). The number of pauses in this group was lower than in the control only in animals at 1 month of age: 2.3 ± 0.22 pauses compared to 3.0 ± 0.20 pauses in the control ($t = 2.39$; $p = 0.018$) (Fig. 2).

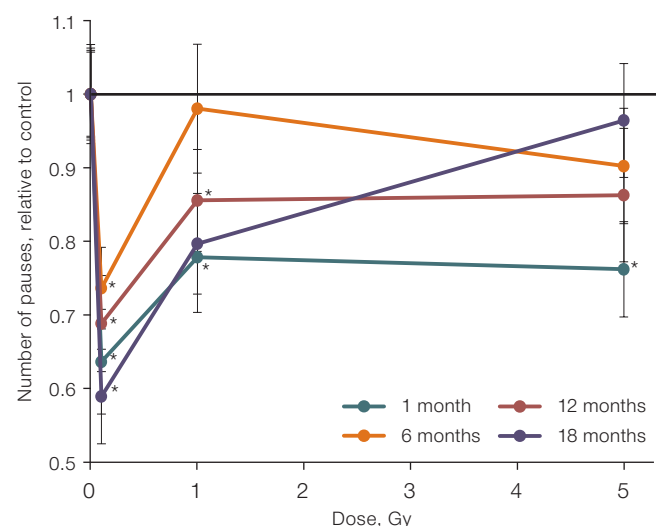


Figure prepared by the authors based on original data

Fig. 2. Change in the number of pauses during raised-beam walking in mice exposed to fractionated gamma radiation at different doses during early development: * — level of statistical significance of differences compared to the non-exposed control group

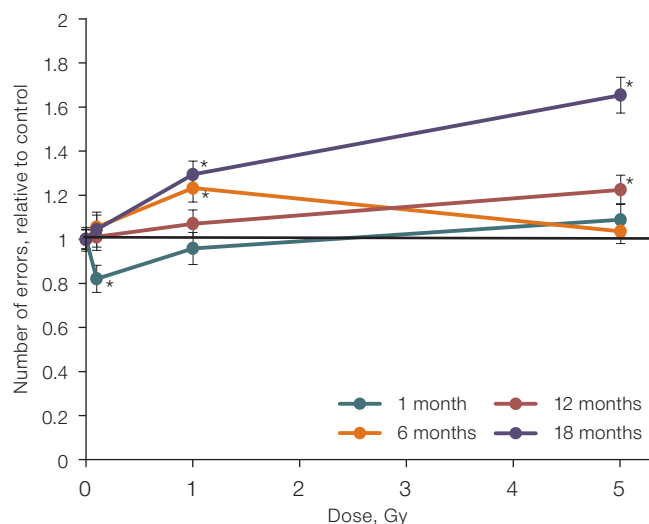


Figure prepared by the authors based on original data

Fig. 3. Change in the number of errors during raised-beam walking in mice exposed to fractionated gamma radiation at different doses during early development:

* — level of statistical significance of differences compared to the non-exposed control group

Conversely, in the long term after exposure to a dose of 5 Gy, the number of errors in performing the test was statistically significantly increased in mice at 12 months: 10.6 ± 0.63 errors versus 8.7 ± 0.37 errors in the control ($t = 2.68$; $p = 0.008$). At 18 months: 12.7 ± 0.61 errors versus 7.9 ± 0.43 errors in the control ($t = 6.52$; $p < 0.001$) (Fig. 3). At 18 months, the number of limb slips from the beam also increased to 0.72 ± 0.13 as compared to a control value of 0.36 ± 0.05 ($t = 3.05$; $p = 0.002$) (Fig. 4). These changes indicate a deterioration in sensorimotor parameters in animals during the long-term period following the completion of fractionated irradiation.

Thus, at the final examination time point (18 months of age), signs of diminished coordination capacity were observed in animals irradiated with a cumulative dose of 5 Gy: although the total walking time did not differ from that of non-exposed animals, mice in the “5 Gy” group made a statistically significant greater number of errors and exhibited more limb slips from the beam. These alterations can be interpreted as indicative of earlier age-related decline in sensorimotor function compared to the control group.

Pairwise comparisons of parameters normalized for sex and age across different dose groups were performed using *post-hoc* analysis via a one-way ANOVA with Bonferroni correction. It was found that for the criteria of walking time and number of pauses, the “0.1 Gy” group was statistically significantly different from all other experimental groups ($p < 0.001$), while the “1 Gy” and “5 Gy” groups showed no significant differences from each other in these parameters. Regarding the number of errors, the “0.1 Gy” group was statistically significantly different from the “1 Gy” and “5 Gy” groups

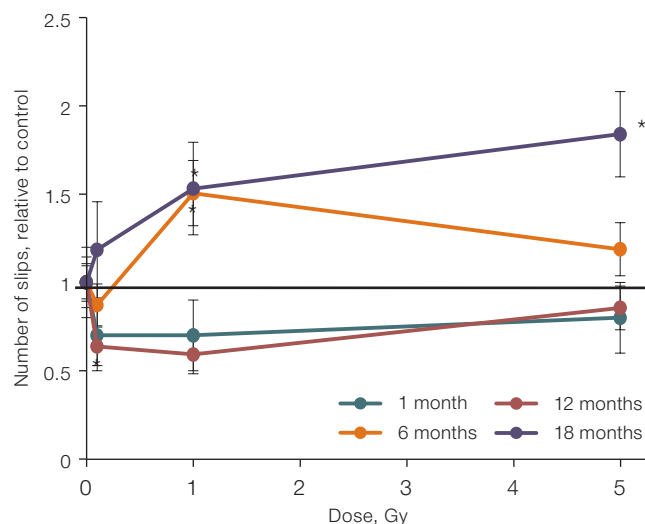


Figure prepared by the authors based on original data

Fig. 4. Change in the number of slips during raised-beam walking in mice exposed to fractionated gamma radiation at different doses during early development:

* — level of statistical significance of differences compared to the non-exposed control group

($p < 0.001$), but did not differ from the non-exposed animals. Conversely, there were no significant differences in this parameter between the “5 Gy” and “1 Gy” groups. The standardized number of slips in the “0.1 Gy” group was lower than in the “5 Gy” group ($p = 0.039$).

Correlation analysis revealed the presence of a moderate direct correlation between various parameters of the raised-beam walking test. Specifically, a moderate direct correlation was demonstrated between walking time and the number of pauses ($r = 0.67$; $p < 0.001$); a correlation was also identified between age and total walking time ($r = 0.32$; $p < 0.001$). The obtained data verify the suitability of the employed tool for assessing coordination in animals. When coordination is impaired, the animal grips the beam tightly apparently fearing a fall, which prolongs the walking time. Conversely, the better the animal’s coordination, the shorter the total walking time, and the fewer errors, slips, and pauses it makes.

The investigations of sensorimotor function in mice exposed to radiation during early development discussed in this paper constitute part of a larger experiment assessing various parameters of higher nervous activity and brain status following fractionated irradiation. For instance, at the age of 2 months, we identified enhanced cognitive function (spatial learning) in the Barnes maze in animals exposed to a cumulative dose of 0.1 Gy [16]. Furthermore, over the course of their lifespan, these same mice exhibited more anxious, neophobic behavior in a marble burying test [17]. Immunohistochemical analysis of the hippocampal dentate gyrus in mice exposed to 0.1 Gy revealed significant stimulation of neurogenesis (elevated levels of PROM1⁺ cells), as well as an increase in the proportion and number of GAP43⁺ cells

expressing neuromodulin, a protein associated with synaptogenesis [16]. It is plausible that the observed alterations in neurogenesis and synaptic plasticity, along with the stimulation of spatial learning, more pronounced neophobic behavior, and increased anxiety in animals exposed to 0.1 Gy, may also be related to the described increase in the speed of movement along the elevated, narrowing beam.

Some studies have reported impaired animal coordination in the inclined beam walking test immediately following fractionated irradiation (5 fractions of 0.1 Gy of X-ray irradiation daily, cumulative dose 0.5 Gy) [18], which was also accompanied by increased anxiety and locomotor activity in the open field test. Our study did not detect early coordination impairments following irradiation at cumulative doses of 0.1–5 Gy. However, it is important to consider the different, more protracted fractionation regimen with the lower doses per fraction used in our work.

Over a longer-term period, other authors have described alterations in sensorimotor function detected by the acoustic startle response test: following single irradiation at 10 weeks of age with a dose of 0.5 Gy, animals exhibited a decreased response speed to an acoustic stimulus; this effect persisted until 12 months of age; at 12 and 18 months, these animals showed reduced locomotor activity in the open field test [19]. Conversely, while irradiation at a low dose (0.063 Gy) did not induce early effects, at 18 months of age, an increase in exploratory activity in the open field and an improved acoustic startle response compared to control animals were demonstrated. These data are consistent with the effects of low (0.1 Gy) and high (5 Gy) dose irradiation observed in our study.

Overall, the neuroprotective effect of low-dose irradiation based on criteria such as reduced pro-inflammatory microglial activation, enhanced cognitive function, and stimulated neurogenesis has been demonstrated in a number of studies [10]. Regarding high-dose irradiation, the primary mechanisms underlying cognitive decline

are considered to be the disruption of adult neurogenesis in the neurogenic niches of the brain (in the hippocampus and cerebellum), pro-inflammatory microglial activation, and impairment of redox status, energy metabolism, and mitochondrial function [2]. Alongside cognitive changes, these same effects could potentially also lead to impaired coordination. For instance, although this particular study did not assess motor function in the irradiated animals, there is evidence of reduced neurogenic activity and the development of neuroinflammation in the cerebellum of rats following fractionated high-dose irradiation [20]. As a continuation of our analysis, it would be advisable to conduct histological examinations of the cerebellum and motor cortex in animals subjected to fractionated irradiation at cumulative doses of 0.1 Gy and 5 Gy during the long-term period post-exposure, to identify morphological correlates of the observed behavioral changes.

CONCLUSION

The study investigated alterations in sensorimotor parameters characterizing coordination in laboratory animals subjected to fractionated gamma radiation at different doses during early development. Fractionated gamma irradiation induces multidirectional, dose-dependent changes in sensorimotor function in mice, which are observed both in the short-term and long-term periods. Immediately following the completion of fractionated irradiation, a stimulatory effect of exposure at a cumulative dose of 0.1 Gy on sensorimotor parameters in mice was demonstrated. This observed improvement in coordination following 0.1 Gy exposure persisted up to the age of 18 months. In the case of irradiation at a cumulative dose of 5 Gy, signs of impaired motor coordination in mice were identified for the first time in this experiment during the long-term period post-exposure, whereas no signs of sensorimotor function disruption were detected within the first 6 months after irradiation.

References

1. Ushakov IB. Current Achievements and Relevant Tasks of Space Radiobiology. *A.I. Burnasyan Federal Medical Biophysical Center Clinical Bulletin*. 2022;2:26–33 (In Russ.). <https://doi.org/10.33266/2782-6430-2022-2-26-33>
2. Kumar R, Kumari P, Kumar R. Central nervous system response against ionizing radiation exposure: cellular, biochemical, and molecular perspectives. *Molecular Neurobiology*. 2025;62(6):7268–95. <https://doi.org/10.1007/s12035-025-04712-z>
3. Sharma NK, Sharma R, Mathur D, Sharad S, Minhas G, Bhatia K, et al. Role of ionizing radiation in neurodegenerative diseases. *Frontiers in Aging Neuroscience*. 2018;10:134. <https://doi.org/10.3389/fnagi.2018.00134>
4. Azizova TV, Bannikova MV, Grigoryeva ES, Rybkina VL, Hamada N. Occupational exposure to chronic ionizing radiation increases risk of Parkinson's disease incidence in Russian Mayak workers. *International Journal of Epidemiology*. 2020;49(2):435–47. <https://doi.org/10.1093/ije/dyz230>
5. Laurent O, Samson E, Caër-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>
6. Kinoshita H, Tanaka K, Nakao R, Iso F, Honda S, Tanaka G, et al. Comparison of mental cognitive function of A-bomb survivors and non-A-bomb survivors in Nagasaki. *Psychiatry and Clinical Neurosciences*. 2019;73(9):594. <https://doi.org/10.1111/pcn.12898>
7. Ishihara K, Kato N, Misumi M, Kitamura H, Hida A, Yamada M. Radiation effects on late-life neurocognitive function in childhood atomic bomb survivors: a radiation effects

- research foundation adult health study. *Radiation Research*. 2022;197(4):403–7.
<https://doi.org/10.1667/RADE-21-00122.1>
8. Motamed MR, Fereshtehnejad SM, Abbasi M, Sanei M, Abbaslou M, Meysami S. X-ray radiation and the risk of multiple sclerosis: Do the site and dose of exposure matter? *Medical Journal of the Islamic Republic of Iran*. 2014;28:145.
 9. Lopes J, Leuraud K, Klovov D, Durand C, Bernier MO, Baudin C. Risk of developing non-cancerous central nervous system diseases due to ionizing radiation exposure during adulthood: systematic review and meta-analyses. *Brain Science*. 2022;12(8):984.
<https://doi.org/10.3390/brainsci12080984>
 10. Boyd A, Byrne S, Middleton RJ, Banati RB, Liu GJ. Control of Neuroinflammation through Radiation-Induced Microglial Changes. *Cells*. 2021;10(9):2381.
<https://doi.org/10.3390/cells10092381>
 11. Fleming SM, Ekhaton OR, Ghisays V. Assessment of sensorimotor function in mouse models of Parkinson's disease. *JoVE*. 2013;(76):50303.
<https://doi.org/10.3791/50303>
 12. Curzon P, Zhang M, Radek RJ, Fox GB. The behavioral assessment of sensorimotor processes in the mouse: acoustic startle, sensory gating, locomotor activity, rotarod, and beam walking. In: Buccafusco JJ, editor. *Methods of Behavior Analysis in Neuroscience*; 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2009.
 13. Atamanyuk NI, Obvintseva NA, Peretykin AA, Andreev SS, Tyukhay MV, Styazhkina EV, et al. Effect of fractionated gamma irradiation on sensorimotor indices in mice in the early postnatal period. *Radiation Safety Problems*. 2025;2(118):69–75 (In Russ.).
 EDN: [ADDTOR](#)
 14. Luong TN, Carlisle HJ, Southwell A, Patterson PH. Assessment of motor balance and coordination in mice using the balance beam. *JoVE*. 2011;(49):2376.
<https://doi.org/10.3791/2376>
 15. Notova SV, Kazakova TV, Marshinskaya OV. Modern methods and equipment for assessing the behavior of laboratory animals (review). *Animal Husbandry and Fodder Production*. 2018;101(1):106–15 (In Russ.).
 EDN: [OYSMIX](#)
 16. Atamanyuk NI, Obvintseva NA, Tukhay MV, Andreev AI, Pryakhin EA. Effect of fractionated gamma irradiation on cognitive function in mice of the first month of life. *Radiation and Risk*. 2024;33(4):107–18 (In Russ.).
<https://doi.org/10.21870/0131-3878-2024-33-4-107-118>
 17. Atamanyuk NI, Obvintseva NA, Peretykin AA, Pryakhin EA. The dose-dependent effect of fractionated γ -radiation on anxiety-like behavior in neonatal mice. *Bulletin of Experimental Biology and Medicine*. 2024;176(6):727–30.
<https://doi.org/10.1007/s10517-024-06097-w>
 18. Koturbash I, Jadavji NM, Kutanzi K, Rodriguez-Juarez R, Kogosov D, Metz GAS, et al. Fractionated low-dose exposure to ionizing radiation leads to DNA damage, epigenetic dysregulation, and behavioral impairment. *Environmental Epigenetics*. 2017;2(4):dvw025.
<https://doi.org/10.1093/eep/dvw025>
 19. Ung MC, Garrett L, Dalke C, Leitner V, Dragosa D, Hladik D, et al. Dose-dependent long-term effects of a single radiation event on behaviour and glial cells. *International Journal of Radiation Biology*. 2021;97(2):156–69.
<https://doi.org/10.1080/09553002.2021.1857455>
 20. Zhou K, Boström M, Ek CJ, Li T, Xie C, Xu Y, et al. Radiation induces progenitor cell death, microglia activation, and blood-brain barrier damage in the juvenile rat cerebellum. *Science Reports*. 2017;7:46181.
<https://doi.org/10.1038/srep46181>

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FEATURES OF THE OVERALL MORTALITY DYNAMICS IN THE POPULATION OF OZERSK URBAN DISTRICT DURING THE COVID-19 PANDEMIC

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Introduction. The pandemic spread of COVID-19, observed in 2020–2021, had an impact on overall mortality among the population. Of particular interest to researchers is the study of trends in overall mortality rates among nuclear industry workers exposed to occupational radiation, as well as among the population living near nuclear facilities.

Objective. Analysis of the overall mortality dynamics among the population of Ozersk — a nuclear industry city — during the COVID-19 pandemic, depending on the combined effect of radiation and non-radiation risk factors.

Materials and methods. A retrospective cohort study was conducted among residents of the Ozersk Urban District (OUD) who died from various causes during the period 2020–2023. Annual reports from Rosstat and the municipal statistics department were analyzed. Data on COVID-19 incidence were provided by the Center for Hygiene and Epidemiology No. 71 in Ozersk. The assessment of survival function depending on the presence of COVID-19, as well as the influence of age and occupational external radiation dose among PA «Mayak» workers, was performed using the Kaplan–Meier method. The analysis of the impact of COVID-19 incidence, adjusted for occupational radiation dose, on the all-cause mortality rate across different age groups was carried out using the Cox proportional hazards model.

Results. Based on the analysis of overall mortality trends for the period 2013–2023, a period associated with the peak of pandemic activity was identified. The impact of COVID-19 on all-cause mortality during the pandemic period was revealed, depending on attained age and occupational radiation dose ($p < 0.05$). The effect of COVID-19 incidence on overall mortality was modified by several risk factors, among which attained age ($p < 0.001$) and accumulated external radiation dose ($p = 0.03$) exerted a significant influence.

Conclusions. COVID-19 incidence during the pandemic period had both direct and indirect effects on overall mortality among the population of Ozersk. Alongside the direct impact of COVID-19, age, and external radiation dose among PA «Mayak» workers, the combination of these factors is also important when assessing the dynamics of overall mortality during the pandemic. The obtained results are of interest for predicting the consequences of potential future pandemic situations and for developing effective strategies to protect the health of populations in nuclear industry cities.

Keywords: pandemic; COVID-19; all-cause mortality; occupational exposure; risk

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ОСОБЕННОСТИ ДИНАМИКИ ОБЩЕЙ СМЕРТНОСТИ НАСЕЛЕНИЯ ОЗЕРСКОГО ГОРОДСКОГО ОКРУГА В ПЕРИОД ПАНДЕМИИ COVID-19

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Введение. Пандемическое распространение заболеваемости COVID-19, наблюдавшееся в период с 2020 по 2021 год, оказало влияние на общую смертность среди населения. Особый интерес для исследователей приобретает изучение динамики показателя общей смертности среди работников атомной отрасли, подвергающихся профессиональному радиационному воздействию, и населения, проживающего вблизи объектов атомной промышленности.

Цель. Анализ динамики общей смертности населения города атомной промышленности Озерск в период пандемии COVID-19 в зависимости от совокупного влияния факторов риска радиационной и нерадиационной природы.

Материалы и методы. Проведено ретроспективное когортное исследование жителей Озерского городского округа (ОГО), умерших от различных причин в период 2020–2023 гг. Анализировали ежегодные отчеты Росстата и муниципального отдела статистики. Данные о заболеваемости от COVID-19 предоставлены Центром гигиены и эпидемиологии № 71 г. Озерска. Оценка функции дожития в зависимости от наличия заболевания COVID-19, а также влияния возраста и дозы внешнего профессионального облучения работников ПО «Маяк» выполнена с использованием метода Каплана – Майера. Анализ влияния заболеваемости COVID-19 с учетом дозы профессионального облучения на интенсивность смертности от всех причин среди различных возрастных групп выполнен методом пропорциональных рисков Кокса.

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Результаты. На основании анализа динамики показателей общей смертности за 2013–2023 годы определен период, связанный с пиком пандемической активности. Выявлено влияние заболевания COVID-19 ($p < 0,05$) в зависимости от достигнутого возраста и дозы профессионального облучения на смертность от всех причин в течение пандемического периода. Влияние заболеваемости COVID-19 на общую смертность модифицировалось воздействием нескольких факторов риска, из которых значимое влияние оказывали достигнутый возраст ($p < 0,001$) и накопленная доза внешнего облучения ($p = 0,03$).

Выводы. Заболеваемость COVID-19 в пандемический период оказывала как прямое, так и опосредованное влияние на общую смертность среди населения Озерска. Наряду с прямым влиянием заболевания COVID-19, возраста и дозы внешнего облучения работников ПО «Маяк» большое значение при оценке динамики общей смертности в период пандемии имеет сочетание этих факторов. Полученные результаты представляют интерес для прогнозирования последствий, связанных с возникновением возможных пандемических ситуаций, и выработки эффективных стратегий по охране здоровья населения городов атомной промышленности.

Ключевые слова: пандемия; COVID-19; общая смертность; профессиональное облучение; риск

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Соответствие принципам этики: не требовалось одобрение биоэтического комитета, поскольку исследование выполнено на основе обезличенных данных медицинских карт (архивные материалы).

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INTRODUCTION

The global spread of the novel SARS-CoV-2 coronavirus infection led to the COVID-19 pandemic, which resulted in an increase in the all-cause mortality rate among the population both in Russia and globally compared to previous years [1, 2]. The main risk factors influencing all-cause mortality during the pandemic, aside from COVID-19 itself, are considered to be attained age, male sex, the presence of comorbid conditions, and racial/ethnic disparities [3].

A distinctive feature of all-cause mortality in the population of the nuclear industry city of Ozersk is chronic exposure to ionizing radiation among employees of the Mayak Production Association (Mayak PA) [4]. Numerous studies have established the influence of ionizing radiation on the risk of mortality from malignant neoplasms and other causes [5–7], which may be considered an additional risk factor affecting mortality rates during the pandemic.

A review of scientific publications in domestic and international journals indexed in the eLibrary and PubMed databases revealed no studies assessing the risk associated with the impact of COVID-19, while accounting for other risk factors including occupational radiation doses, on all-cause mortality rates during the pandemic.

The aim of the study is to analyze the dynamics of overall mortality among the Ozersk population, a nuclear industry city, during the COVID-19 pandemic, depending on the combined effect of radiation-related and non-radiation-related risk factors.

MATERIALS AND METHODS

A retrospective cohort study among residents of the Ozersk urban district (OUD) who died from various causes in 2020–2023 was conducted. The crude all-cause mortality rate was calculated using official statistical data for the period 2013–2023.^{1,2} The all-cause mortality rate (μ) per 1000 individuals (‰) was calculated according to the standard methodology described in [1]. The mortality risk intensity analysis was performed in the cohort of individuals who were alive at the date of the COVID-19 pandemic declaration (11.03.2020) and who died from different causes during the subsequent three-year period.

The source of information for this study was data on COVID-19 morbidity and mortality provided by the Center for Hygiene and Epidemiology No. 71 in Ozersk. Data on external occupational radiation dose for employees of the Mayak Production Association (Mayak PA) were obtained from the Mayak Worker Registry [5, 6]. The Kaplan–Meier survival function for individuals who died from all causes during the period 2020–2023 was estimated based on the attained age at the time of death [8]. To characterize the attained age at the onset of observation, a categorical variable was used, comprising the following age intervals: 0–19 years, 20–39 years, 40–59 years, 60–79 years, and 80 years and older. The use of broad age intervals was implemented to increase statistical power for groups with a small number of observations ($n < 30$).

Based on employment status at the main or auxiliary production units of Mayak PA in 1948–2016, the

¹ Socio-Economic Development Statistics for Ozersk Urban District <http://ozerskadm.ru/regulatory/passport/> (access data 22.02.2025).

² Federal State Statistics Service (Rosstat) <http://rosstat.gov.ru/> (access data 22.02.2025).

OUN population was categorized into two groups: “general population” and “occupationally exposed” population. Data on the cumulative effective dose of external gamma radiation for the occupationally exposed persons were obtained using the Mayak Worker Registry [10]. A comparative analysis of mortality risk intensity across different age groups within the population, considering occupational radiation dose, was conducted using the Cox proportional hazards model [11]. The all-cause mortality hazard (h) was defined by the following equation:

$$h_t = h_0(t) \times e^{\beta_i x_i}, \quad (1)$$

h_t — all-cause mortality hazard at time t ; $h_0(t)$ — baseline hazard at time t ; β_i — regression coefficient; x_i — the value of the covariate determining the risk factor.

The regression model was developed by sequentially adding predictors to the base model. The Likelihood Ratio Test (LRT) was used to compare the base and extended models. The proportional hazards assumption was verified using the Cox proportional hazards test, with a critical p -value of 0.05 for rejecting the null hypothesis that the proportional hazards assumption is violated. The statistical significance of the regression coefficients was assessed using the Wald Chi-square test. Multicollinearity among parameters was checked by calculating the Variance Inflation Factor (VIF) using formula (2):

$$VIF = \frac{1}{1 - R_j^2}, \quad (2)$$

where R^2 — coefficient of determination for the j -th variable regression.

The permissible multicollinearity threshold for the j -th variable in the model was set at $VIF \leq 5$. The statistical significance of differences between survival functions for different risk factors was assessed using the non-parametric log-rank test [9]. The conventional significance level of $\alpha = 0.05$, corresponding to a 95% confidence interval, was used to determine statistical significance. Results were considered statistically significant at $p < 0.05$.

RESULTS

In this study, crude all-cause mortality rates (Crude Mortality Rate, CMR) were calculated for the OUN and the Russian Federation for the period 2013–2023, based on population size and number of deaths from all causes. The dynamics of the crude all-cause mortality rate in the OUN compared to the Russian Federation data over the period 2013–2023 are presented in Figure 1.

It was established that the dynamics of the all-cause mortality rate in the OUN in 2020–2023 followed a pattern similar to that of the Russian Federation. However, the all-cause mortality rate in the OUN was consistently

higher than the corresponding rates for the Russian Federation since 2016 and throughout the pandemic period (the maximum discrepancy was observed in 2021: 17.2‰ for the Russian Federation and 18.3‰ for Ozersk) (Fig. 1). The higher all-cause mortality rate in Ozersk compared to the Russian Federation data, when comparing unstandardized rates, may be a consequence of differences in the age distribution of the populations.

A statistically significant excess in the all-cause mortality rate in the OUN relative to its pre-pandemic average (13.7‰) [2] was recorded over the three-year period of 2020–2022. A pronounced deviation of the OUN all-cause mortality rate from its expected value was observed in 2020 at 16.2‰, peaked in 2021 (18.3‰), followed by a sharp decline in 2022 to 14.6‰, returning to pre-pandemic levels (12.7‰) in 2023. These findings indicate the necessity for a more detailed examination of the 2020–2022 period, which corresponds to the peak pandemic activity.

For this purpose, a cohort of OUN residents who were alive at the pandemic declaration date and died from all causes during the period 11.03.2020–31.12.2022 was selected. The distribution of the study cohort by sex, age, presence of COVID-19 as the underlying cause of death, and cumulative external gamma radiation dose intervals among the personnel is presented in Table 1.

The presented data indicate an almost equal distribution of male and female subjects among those who died from all causes during the pandemic period. The largest proportion of all-cause deaths during the pandemic period (81.4%) corresponded to an attained age over 60 years. The mean attained age at the onset of observation was 70.9 ± 0.25 years: for male subjects — 65.9 ± 0.35 years, for female subjects — 75.8 ± 0.32 years. The mean age at death in the OUN population during the pandemic period was

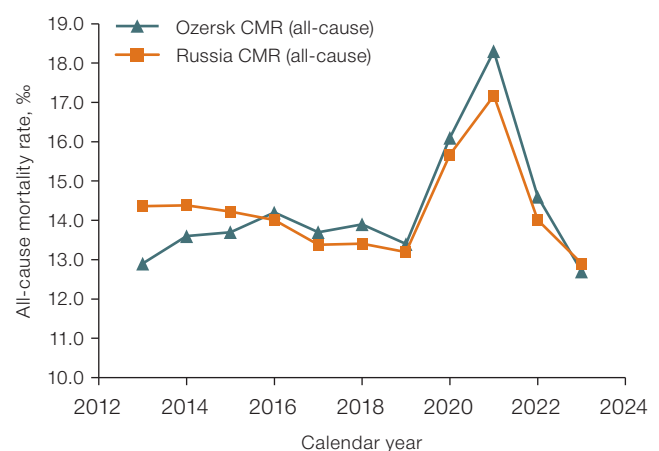


Figure prepared by the author

Fig. 1. All-cause mortality rates (CMR) in Russia and the Ozersk urban district for the 2013–2023 period

Table 1. Characteristics of the cohort of OUD residents who died during the pandemic period, by attained age, sex, external occupational radiation dose, and COVID-19 status

Parameter	Absolute number	Proportion, %	COVID-19 (%)*
Gender			
Male	1961	49.4	459 (46.3)
Female	2008	50.6	532 (53.7)
Attained age, years			
0–19	21	0.5	3 (0.3)
20–39	188	4.7	30 (3.0)
40–59	638	16.1	135 (13.6)
60–79	1695	42.7	477 (48.1)
80+	1427	36.0	346 (34.9)
Cumulative dose, mSv			
0	117	2.9	23 (2.3)
0–9	389	9.8	100 (10.1)
10–99	339	8.5	78 (7.9)
100+	63	1.6	14 (1.4)
Not measured (general population)	3,061	77.1	776 (78.3)
Underlying cause of death			
U07.1, U07.2	425	10.7	370 (37.3)
Other causes	3421	89.3	621 (62.7)
Total	3969	100.0	991 (25.0)

Table prepared by the author

Note: * — the percentage is calculated from the total number of cases in each subgroup.

72.5 ± 0.32 years: for male subjects — 67.5 ± 0.35 years, for female subjects — 77.3 ± 0.32 years. The proportion of the deceased for whom the underlying cause of death was COVID-19 (including individuals without SARS-CoV-2 identification) was 10.7%.

In the study cohort, the proportion of COVID-19 cases accumulated during the pandemic period was 25%, being higher among the female subjects (53.7%). The distribution by attained age reached a maximum of 48.1% among individuals aged 60–80 years. The cumulative proportion of individuals who was diagnosed with COVID-19 during the pandemic period and were exposed to external gamma radiation was 21.7%. The proportion of individuals who sought medical care for COVID-19 during the pandemic period, for whom this disease became the underlying cause of death, was 37.2%.

The all-cause mortality in the OUD population, stratified by age, was assessed using the Kaplan–Meier survival function for the age intervals of 0–39 years,

40–59 years, 60–79 years, and 80 years and older. The dynamics of the all-cause mortality among the OUD population across age intervals are presented in Figure 2.

The survival function represents the empirically observed proportion (%) of individuals who survived to a specific observation time point, depending on their attained age. The data presented in Figure 2 indicate a change in the overall mortality rate among the population across different age groups, which was more pronounced during the first two years of the pandemic period (2020–2021). The working age (40–59 years) group exhibited the lowest all-cause mortality rates throughout the entire pandemic period. Pairwise comparison of survival functions using the log-rank test revealed statistically significant differences ($p = 0.012$) between the age subgroup 19–39 years (mean attained age 34 ± 0.33 years) and 40–59 years (mean attained age 51.7 ± 0.22 years).

The number of Mayak PA employees who died during the study period was 908 (22.9%). The mean

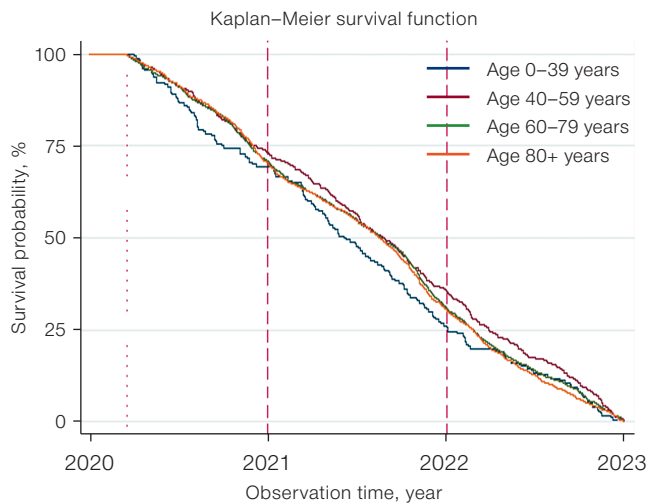


Figure prepared by the author

Fig. 2. Survival function for different age subgroups in the OUD population who died during the pandemic period: the date (11.03.2020) is indicated by points

cumulative external gamma radiation dose for workers (including 117 individuals with zero measured doses) was 27.6 ± 1.97 mSv (median 7.11 mSv, maximum 547.05 mSv). The Kaplan-Meier survival function was constructed for the general population ("Population") and occupationally exposed persons ("Workers") categories. The dynamics of all-cause mortality in the OUD population, stratified by the population subgroups, are presented in Figure 3.

The data presented in Figure 3 indicate observed slight differences in overall mortality rates among the personnel of PA «Mayak» compared to the general population during the peak of the coronavirus infection spread in 2021. A comparison of survival functions among personnel for marginal categories of accumulated external radiation dose (measured dose value of 0 mSv and individuals who accumulated a dose exceeding 100 mSv)

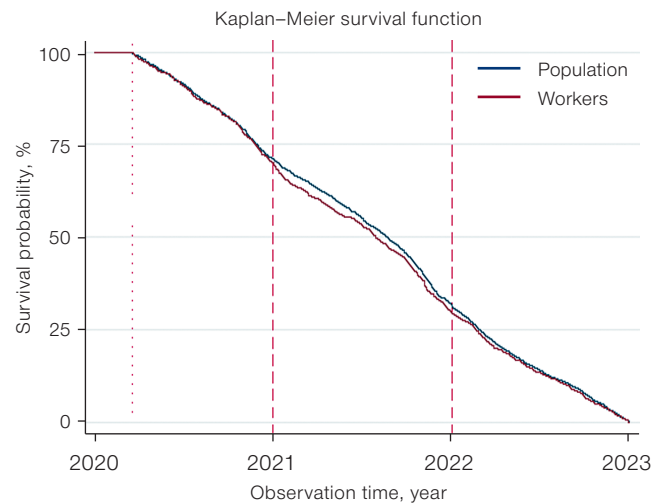


Figure prepared by the author

Fig. 3. Survival function for deceased individuals in the OUD during the pandemic period, stratified by the presence of exposure to external occupational radiation

using the log-rank test showed borderline statistical significance of differences in all-cause mortality rates during the pandemic ($p = 0.053$).

The results of the regression analysis of all-cause mortality hazard, considering age, sex, COVID-19 status, and cumulative external gamma radiation dose, performed using a multivariate Cox regression model, are presented in Table 2.

The model parameter estimates indicate that the influence of sex is not a linear predictor when comparing all-cause mortality during the pandemic period. The external gamma radiation dose, included in the model as a linear predictor, had a statistically significant effect on the outcome measure ($p = 0.033$), amounting to 0.1% per 1 mSv. Adjusting for the presence of COVID-19 (U07.X) increased the statistical significance of the resulting coefficient ($p = 0.01$) and changed the overall mortality rate

Table 2. Results of the all-cause mortality hazard modeling, adjusted for age, sex, and cumulative external radiation dose (95% confidence interval)

Parameter	Coefficient	Standard error	<i>p</i>	[95% CI]	
Gender (male)	1.064	0.034	0.062	0.997	1.137
Age (20-year categories)	1.093	0.022	<0.001	1.050	1.138
Cumulative dose, mSv	1.001	0.0005	0.033	1.0001	1.002
COVID-19	4.942	0.20	<0.001	4.560	5.357
Variable interaction					
COVID-19	1.424	0.228	0.027	1.041	1.948
Influence of age and COVID-19	1.494	0.074	<0.001	1.356	1.647
Influence of dose and COVID-19	1.002	0.001	0.050	0.999	1.004

Table compiled by the author

by 1%. This observation suggests that chronic exposure to ionizing radiation among employees of PA «Mayak» was a factor that, during the pandemic, was more specific to causes of death not related to COVID-19.

The all-cause mortality hazard increased by a factor of 1.001 with each 1 mSv increase in cumulative external radiation dose. Based on the predictive estimates obtained from the model, for Mayak PA workers exposed to a dose of 27.6 mSv (the mean value in the study cohort), the all-cause mortality hazard increased by 2.8% compared to the non-exposed subgroup. The predicted effect of the cumulative external gamma radiation dose on mortality intensity during the pandemic period among Mayak PA workers hired at main and auxiliary productions in 1948–2000 is presented in Figure 4.

Cox model risk estimates (Fig. 4) show that the actual accumulated levels of external radiation doses among workers hired for production in 1948–2010 and who survived until the date of the pandemic declaration (mean: 27.6 ± 1.97 mSv) led to a statistically significant, but quantitatively small change in the all-cause mortality hazard rate.

For risk prediction purposes, the impact of high external radiation doses of 1 Sv, typical for the initial operational period of PA «Mayak» (1948–1957), on the all-cause mortality hazard rate during the pandemic was modelled. The simulation results showed that if all members of the study cohort, as of the entry date into follow-up, had been able to accumulate an external occupational gamma radiation dose of 1 Sv (indicated by a dotted line in Figure 4), the effect of the external radiation dose on the overall mortality rate during the pandemic would have been comparable to the effect of having a COVID-19 diagnosis (Fig. 5).

The obtained results indicate that COVID-19 is a significant predictor increasing the all-cause mortality hazard rate among the population of the Ozersk Urban District (OUD), and is modified by the influence of occupational external radiation dose and attained age. The assessment of the direct effect of COVID-19 shows a 4.9-fold increase in the outcome measure within the cohort if a patient has had at least one episode of the disease requiring medical care.

Including an interaction term for COVID-19 status and attained age in the model revealed that the direct effect of the disease «masks» the influence of age (Table 2). For every 20 years of increased attained age, the effect of having COVID-19 increases the all-cause mortality hazard rate by 49 %, while the influence of the direct effect remains statistically significant but decreases to 1.42 ($p < 0.05$).

Including the interaction between occupational radiation dose and COVID-19 in the model revealed that, compared to the non-exposed general population, the all-cause mortality hazard among exposed individuals with a history of COVID-19 was higher by 0.2%. However, this coefficient had borderline statistical significance ($p = 0.05$), indicating a potential non-linearity of this interaction.

DISCUSSION

The study did not use data characterizing the spread dynamics of the novel coronavirus infection («pandemic waves»), differences in virus strains, as well as the number of infected individuals who had a mild form of the disease and did not seek medical care.

One of the confounding factors affecting the comparative assessment of the crude overall mortality rate

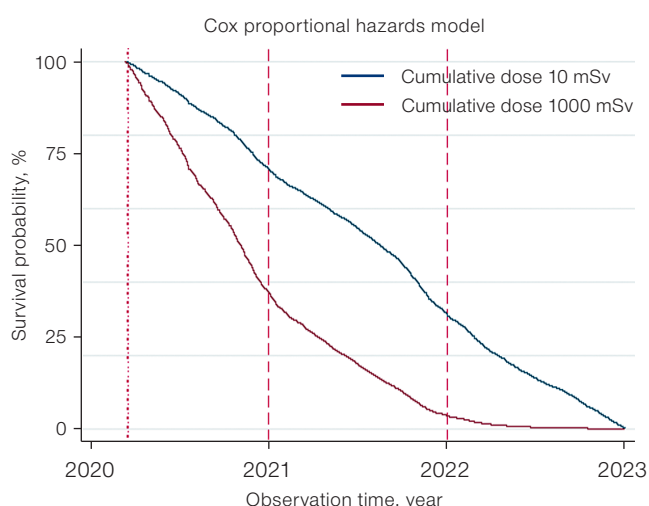


Figure prepared by the author

Fig. 4. Predictive estimates of cause-specific mortality hazard during the pandemic period 2020–2023 for Mayak PA personnel, by cumulative external gamma radiation dose

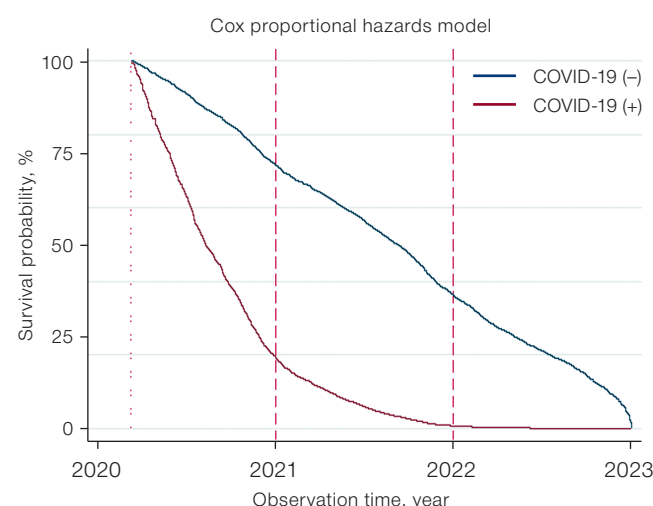


Figure prepared by the author

Fig. 5. Predictive estimates of cause-specific mortality hazard during the pandemic period 2020–2023, stratified by the presence or absence of a confirmed COVID-19 disease

against national statistics is the difference in life expectancy between the population of the Ozersk Urban District and that of Russia [12].

A specific risk factor influencing the all-cause mortality rate is chronic radiation exposure experienced by personnel of nuclear industry facilities. The impact of occupational radiation exposure on the risk of death from diseases other than cancer has been widely studied recently [7, 13]. The results of the present study indirectly support the hypothesis of an association between ionizing radiation exposure and the risk of non-cancer mortality.

The structure of all-cause mortality during the pandemic period, beyond deaths directly from COVID-19 as the underlying cause (U07.1, U07.2), includes various clusters of diseases, such as malignant neoplasms (C00–C97). The influence of radiation, considered a potential cause of oncological diseases in the exposed occupational cohort [5], may explain the observed increase in all-cause mortality in the study cohort after excluding deaths from COVID-19 from the analysis.

Potential limitations of this study include the duration of the observation period, which encompasses only the first three years following the declaration of the COVID-19 pandemic. Despite the fact that the pandemic officially lost its status as a public health emergency in 2022, the presence of the SARS-CoV-2 infectious agent in the population [14] remains a relevant issue for monitoring, given the potential long-term consequences of COVID-19, whose impact on mortality risk has not yet been sufficiently studied.

For a deeper understanding of the pandemic's impact mechanisms within the context of public and occupational health in nuclear industrial complexes, a detailed examination of the relationship between radiation risk factors and specific causes of death is necessary. Furthermore, investigating the patterns of COVID-19's influence on the risk of death from malignant neoplasms is a relevant issue in international research [15]. Given the increased probability of developing lung malignant neoplasms among workers in plutonium production facilities

[16], a promising direction for future research is the study of the association between the dose from internal exposure to incorporated ^{239}Pu and lung cancer mortality during the pandemic period.

CONCLUSIONS

According to the World Health Organization's conclusion, the possibility of future pandemic spread of novel coronavirus infection cannot be ruled out. Based on this, analyzing the consequences of the past pandemic is relevant from the perspective of ensuring preparedness for similar situations in order to minimize potential harm to public health.

The results of the conducted study showed that during the pandemic period, the population of the Ozersk — a nuclear industry city — experienced a change in the all-cause mortality hazard rate, depending on the influence of various factors. The groups most affected by the pandemic were young and elderly individuals, as well as workers exposed to occupational external radiation.

The impact of COVID-19 on the all-cause mortality hazard rate occurred through both direct and indirect mechanisms. The most significant modifier of all-cause mortality risk among individuals with COVID-19 was attained age. Alongside the direct effects of COVID-19, age, and external radiation dose among workers at PA «Mayak», the combination of these risk factors is also important when assessing the dynamics of overall mortality during the pandemic.

The presence of a statistically significant modification in the combined effect of COVID-19 and radiation dose indicates the need for further, more detailed study of radiobiological patterns in the progression of pathological processes in individuals exposed to combined risk factors of both radiation and non-radiation nature.

The obtained results are also relevant for predicting the consequences of potential future pandemic situations and for developing effective health protection strategies for occupationally exposed workers.

References

- Osipov MV, Sokolova VA, Kushnir AS. Evaluation of the impact of COVID-19 pandemic on overall mortality in Ozersk urban district. *Extreme Medicine*. 2022;24(2):74–80 (In Russ). <https://doi.org/10.47183/mes.2022.011>
- Osipov MV, Fomin EP. Assessment of COVID-19 pandemic-related detrimental impact on the population of nuclear city population: Two-year results. *Russian Open Medical Journal*. 2023;12:e0101. <https://doi.org/10.15275/rusomj.2023.0101>
- Zhang JJ, Dong X, Liu GH, Gao YD. Risk and Protective Factors for COVID-19 Morbidity, Severity, and Mortality. *Clinical Reviews in Allergy and Immunology*. 2023;64(1):90–107. <https://doi.org/10.1007/s12016-022-08921-5>
- Novoselov VN, Nosach YuF, Entyakov BN. *Atomic heart of Russia*. Ed. Baranov SV. Chelyabinsk: Autograph, 2014 (In Russ.).
- Shilnikova NS, Preston DL, Ron E, Gilbert ES, Vassilenko EK, Romanov SA, 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\[0787:cmrawaj2.0.co;2](https://doi.org/10.1667/0033-7587(2003)159[0787:cmrawaj2.0.co;2)
- Sokolnikov M, Preston D, Gilbert E, Schonfeld S, Koshurnikova N. 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>
- Moseeva MB, Azizova TV, Grigoryeva ES, Haylock R. Risks of circulatory diseases among Mayak PA workers with radiation doses estimated using the improved Mayak Worker Dosimetry System 2008. *Radiation and Environmental Biophysics*. 2014;53(2):469–77. <https://doi.org/10.1007/s00411-014-0517-x>

8. Gomes AP, Costa B, Marques R, Nunes V, Coelho C. Kaplan–Meier Survival Analysis: Practical Insights for Clinicians. *Acta Medica Portuguesa*. 2024;37(4):280–5. <https://doi.org/10.20344/amp.21080>
9. Singh R, Mukhopadhyay K. Survival analysis in clinical trials: Basics and must know areas. *Perspectives in Clinical Research*. 2011;2(4):145–8. <https://doi.org/10.4103/2229-3485.86872>
10. Koshurnikova NA, Shilnikova NS, Okatenko PV. Characteristics of the cohort of workers at the Mayak nuclear complex. *Radiation Research*. 1999;152(4):352–63.
11. Deo SV, Deo V, Sundaram V. Survival analysis—part 2: Cox proportional hazards model. *Indian Journal of Thoracic and Cardiovascular Surgery*. 2021;37(2):229–33. <https://doi.org/10.1007/s12055-020-01108-7>
12. Telnov VI, Tretyakov FD. Dynamics of age and sex structure of the population of Ozersk (1953–2008). *Extreme Medicine*. 2009;4:108–22 (In Russ.).
13. Azizova TV, Muirhead CR, Moseeva MB, Grigoryeva ES, Sumina MV, O'Hagan J, et al. Cerebrovascular diseases in nuclear workers first employed at the Mayak PA in 1948–1972. *Radiation and Environmental Biophysics*. 2011;50(4):539–52. <https://doi.org/10.1007/s00411-011-0377-6>
14. Otto SP, Day T, Arino J, Colijn C, Dushoff J, Li M, et al. The origins and potential future of SARS-CoV-2 variants of concern in the evolving COVID-19 pandemic. *Current Biology*. 2021;31:R918–29. <https://doi.org/10.1016/j.cub.2021.06.049>
15. Chavez-MacGregor M, Lei X, Zhao H, Scheet P, Giordano SH. Evaluation of COVID-19 Mortality and Adverse Outcomes in US Patients with or without Cancer. *JAMA Oncology*. 2022;8(1):69–78. <https://doi.org/10.1001/jamaoncol.2021.5148>
16. Stram DO, Sokolnikov M, Napier BA, Vostrotin VV, Efimov A, Preston DL. Lung Cancer in the Mayak Workers Cohort: Risk Estimation and Uncertainty Analysis. *Radiation Research*. 2021;195(4):334–46. <https://doi.org/10.1667/RADE-20-00094.1>

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COMPUTATIONAL PHANTOM FOR RED BONE MARROW DOSIMETRY IN 15-YEAR-OLD ADOLESCENTS

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Introduction. Computational phantoms are widely used for assessing radiation doses to the red bone marrow (RBM) from bone-seeking radionuclides. Among them, strontium isotopes are the most common. The development of phantoms for ^{89,90}Sr requires accurate description of bone shape, size, and microarchitecture. To date, phantoms for newborns, one-year-old, five-year-old, and 10-year-old children, as well as for adult males and females, have been proposed. This study is a continuation of our work on creating digital skeletal models for humans of different sexes and ages.

Objective. Development of computational phantoms for the skeleton for 15-year-old adolescents with the purpose of assessing doses in RBM from incorporated beta-emitting radionuclides.

Materials and methods. The phantoms were developed using the stochastic parametric skeletal dosimetry (SPSD) approach. Skeletal regions with active hematopoiesis were identified and segmented. The parameters of the segment models were estimated based on literature data, including bone microstructural characteristics, cortical bone layer thickness, bone and segment dimensions, the fractional content of RBM, and the chemical composition and density of the modeled media. The variability ranges of these parameters were also assessed.

Results. The developed phantoms for 15-year-old male and female adolescents comprise 46 segments each; parameters for 14 of these segments differed between males and females. The phantom dimensions ranged 3.5–66 mm; the cortical bone thickness varied 0.3–2.3 mm.

Conclusions. The phantoms developed in this work reflect the dimensions and structure of skeletal regions with active hematopoiesis in 15-year-old adolescents, account for sexual dimorphism, and simulate the variability of skeletal characteristics.

Keywords: trabecular bone; cortical bone; bone marrow dosimetry; computational phantoms; strontium

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ВЫЧИСЛИТЕЛЬНЫЙ ФАНТОМ ДЛЯ ДОЗИМЕТРИИ КРАСНОГО КОСТНОГО МОЗГА ДЛЯ 15-ЛЕТНИХ ЮНОШЕЙ И ДЕВУШЕК

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Введение. Вычислительные фантомы широко применяются для оценки доз облучения красного костного мозга от остеотропных радионуклидов. Наиболее распространенными такими радионуклидами являются изотопы стронция. Создание фантомов для ^{89,90}Sr требует аккуратного описания формы, размеров и микроархитектуры костей. На сегодняшний день опубликованы описания фантомов новорожденного, годовалого, 5-летних и 10-летних детей, а также взрослых мужчины и женщины. Данное исследование является продолжением работы по созданию цифровых моделей скелета для людей разного пола и возраста.

Цель. Разработка вычислительных фантомов скелетов 15-летних юношей и девушек для оценки доз в красном костном мозге (ККМ) от инкорпорированных бета-излучающих радионуклидов.

Материалы и методы. Для создания фантомов был использован СПСД (stochastic parametric skeletal dosimetry) подход. Были выделены участки скелета с активным гемопоэзом, которые были разделены на сегменты. Параметры моделей сегментов были оценены по данным литературы и включали в себя: характеристики микроструктуры кости, толщину слоя кортикальной кости, размеры костей и их участков, а также долю содержания ККМ, химический состав и плотность моделируемых сред. Также были оценены значения вариабельности этих параметров.

Результаты. Разработанные фантомы 15-летних юношей и девушек включают по 46 сегментов; параметры 14 из них различались для юношей и девушек. Размеры фантомов были 3,5–66 мм, а толщина кортикальной кости 0,3–2,3 мм.

Выводы. Фантомы, полученные в рамках данной работы, отражают размеры и структуру участков скелета с активным гемопоэзом 15-летних юношей и девушек, учитывают половые различия, а также имитируют вариабельность характеристик скелета.

Ключевые слова: трабекулярная кость; кортикальная кость; дозиметрия костного мозга; вычислительные фантомы; стронций

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INTRODUCTION

Assessment of doses from incorporated bone-seeking radionuclides plays an essential role in predicting radiogenic risks for individuals residing in contaminated areas. Strontium isotopes are among the most common of these radionuclides, which can be released into the environment both through global fallout and other technogenic radiation incidents. ^{90}Sr is a long-lived, bone-seeking, beta-emitting radionuclide; its accumulation in bone tissue leads to chronic irradiation of the red bone marrow (RBM), thereby increasing the risk of developing leukemia [1–4].

Assessment of doses from strontium isotopes involves the use of biokinetic and dosimetric models. A biokinetic model was previously developed at the Ural Scientific and Practical Center for Radiation Medicine (Russia).¹ This model simulates the transport processes of Sr within the body; the output of this model is the specific activity of Sr in bone tissue. The dosimetric model enables calculation of dose conversion factors (DF) from the specific activity of the radionuclide in bone tissue to the absorbed dose rate in RBM (Gy/s) [5]. Such models are developed based on the creation of computational phantoms, representing 3D models that simulate the spatial arrangement and composition of media in which radiation transport will subsequently be simulated. Developing such phantoms for beta-emitters is a challenging task due to the necessity of adequately describing both the linear dimensions of bones and their microstructure.

Modern phantoms used for the internal dosimetry of strontium are based on the analysis of medical images of human skeletons² [6–10]. Consequently, such phantoms have a significant limitation for their use in cohort studies. Indeed, due to the limited amount of autopsy material, they do not allow for the assessment of uncertainties associated with the variability of skeletal dimensions and microarchitecture within a population.

An alternative approach consists in the parametric method of stochastic modeling of bone structures, referred to as stochastic parametric skeletal dosimetry (SPSD) modeling [11]. This approach, developed at the Ural Scientific and Practical Center for Radiation Medicine (Russia), estimates the phantom parameters

based on published bone measurement results rather than using medical images. The extensive body of literature enables assessment of the variability in skeletal characteristics within a population and the associated DF variability.

Previous publications [12–16] were the first to present the parameters of skeletal phantoms for newborns, one-year-old, 5-year-old, and 10-year-old children, as well as for adult males and females. The skeletal regions with active hematopoiesis are identical in 15-year-olds and adults. Furthermore, many skeletal regions in 15-year-old individuals are similar in shape and microstructural characteristics to those in adults. For this reason, phantoms for 15-year-old male and female adolescents were developed based on adult phantoms; in other words, some parameters for 15-year-old phantoms were set equal to those for adults. Similar to adults, sexual dimorphism assessment is also important for 15-year-olds. Thus, this our study represents a new stage in the development of computational phantoms for different age groups.

In this work, we aim to develop computational phantoms of the skeleton for 15-year-old male and female adolescents for assessing doses in RBM from incorporated beta-emitting radionuclides.

MATERIALS AND METHODS

The stages of phantom development did not differ from those for other age groups [11]. The primary stage involved identifying the modeling objects, i.e., skeletal regions with active hematopoiesis, and estimating the mass fraction of RBM in each such a region. Subsequently, linear dimensions and bone microstructural characteristics were estimated based on literature data. Following this, each modeled skeletal region was subdivided into smaller parts. A computational phantom was generated for each such a part.

The RBM mass fraction in various skeletal bones was estimated based on published data [17]. Within the SPSPD approach, not the entire skeleton is modeled, but only its regions containing RBM. This rule also applies to individual bones; that is, only their parts containing RBM were modeled. Accordingly, based on MRI study results [18–23], the distribution of RBM within the bones was assessed.

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The phantom parameters for 15-year-old male and female adolescents, similar to those for other age groups, included average values of bone microstructural parameters, i.e., trabecular thickness (*Tb. Th.*), mean trabecular separation (*Tb. Sp.*), and the bone volume fraction (*BV/TV*) [24]. Bone and segment dimensions, as well as cortical bone layer thickness (*Ct. Th.*), were also phantom parameters. We estimated all these parameters based on measurement results obtained using histomorphometry and micro-computed tomography (micro-CT) and published in the respective literature.

Literature data were collected and analyzed according to a previously described methodology [24]. The parameters of the adult male and female phantoms [16] were used for those skeletal regions for which no published data were found. Measurement results from bones of Caucasians and Mongoloids were included in the analysis, given that these ethnic groups are representative of the Ural region for the age range of 13–17 years.

Bone microarchitecture parameters were estimated using the published data obtained by histomorphometry and micro-CT. Cortical bone thickness was assessed based on published measurement results obtained using CT, micro-CT, and micrometer gauges. Linear bone dimensions were estimated from published measurement results utilizing anatomical boxes, ultrasonography, radiographic studies, and CT.

In the absence of sexual dimorphism in the studied bone characteristics, datasets for both sexes were pooled. In such cases, the bone segment was modeled independently of sex. In other instances, phantoms for different sexes were modeled separately.

Each skeletal region with active hematopoiesis was subdivided into smaller parts — segments. All segments feature homogeneous bone microarchitecture and cortical layer thickness and are described by simple geometric shapes [25]. A basic phantom bone segment (BPS) was modeled for each segment. The segmentation was performed such that each segment possessed a homogeneous microarchitecture and a homogeneous cortical layer thickness. For BPSs represented by elongated cylinders or elongated/flat rectangular parallelepipeds, only the portion of BPS whose maximum linear dimensions reached 30 mm was modeled. Further increasing the maximum size of such a BPS does not affect the DF [26]. Thus, segmentation permits accounting for heterogeneous microarchitecture and cortical thickness within a bone, reduces the size of the phantoms (thereby enabling higher resolution), and improves simulation accuracy.

The density and chemical composition of the modeled media (mineralized bone and bone marrow) were identical for all BPSs and were estimated based on literature sources [27, 28]. The generation of BPSs was performed using the Trabecula software application developed in our laboratory [29]. A BPS consists of three media: bone marrow (BM) — the target tissue, as well as cortical bone (CB) and trabecular bone (TB) — two separate source tissues.

Trabecular bone was modeled as a 3D network of bone strands — trabeculae — whose spatial arrangement and thickness were randomly selected within the bounds of bone microarchitecture parameter variability estimated from published data [30–34]. Bone marrow is located in the spaces between the trabeculae, and cortical bone (CB) encases the trabecular bone (TB) externally as a continuous layer with a specified thickness. The voxel size for each BPS was adjusted such that it did not exceed 70% of *Tb. Th.* and ranged within 50–140 μm [29]. The volumes of the media comprising the phantom were calculated in the Trabecula software.

Phantoms created using the SPSP approach enable the assessment of DF uncertainties associated with the variability of skeletal characteristics within a population. For this purpose, sets of supplementary phantoms of bone segment (SPS) were modeled for each BPS. While the BPS was modeled with average parameter values, the parameter values for the SPS were randomly sampled within the boundaries of the minimum and maximum measured values of their variability. The method for uncertainty assessment was described in detail in [35].

Skeletal regions with active hematopoiesis in a 15-year-old adolescent, bone segmentation, and cross-sections of individual segments using the clavicle as an example are presented in the figure.

RESULTS

The set of skeletal regions with active hematopoiesis for 15-year-olds did not differ from that for adults; however, there are differences in the RBM distribution among these regions, as demonstrated in Table 1.

Table 1 shows that the skeleton of 15-year-olds comprises 12 skeletal regions with active hematopoiesis, with the RBM mass fraction ranging 1.0–18.6%. No sexual dimorphism was observed in the RBM distribution within the skeleton.

The elemental composition of the modeled media is equivalent to that in adults [16] and was estimated based on published data [28]. We used the measurement results published for the density of the cortical bone layer in 15-year-olds [27] as the density for both CB and TB (1.85 g/cm^3). The BM density was assumed to be 1.0 g/cm^3 [28].

No significant sexual dimorphism was found in bone microarchitecture characteristics [26]; therefore, data from males and females were pooled. Table 2 presents the microarchitecture parameter values for the BPS of 15-year-old male and female adolescents.

The variability ranges of bone microarchitecture parameters within a bone were used to set the characteristics of the 3D trabecular network model, thereby enhancing its realism. For 15-year-old adolescents, no such variability values were found; therefore, these values were assumed to be the same as for adults [36].

The linear dimensions and cortical layer thickness adopted for the BPS of 15-year-old male and female

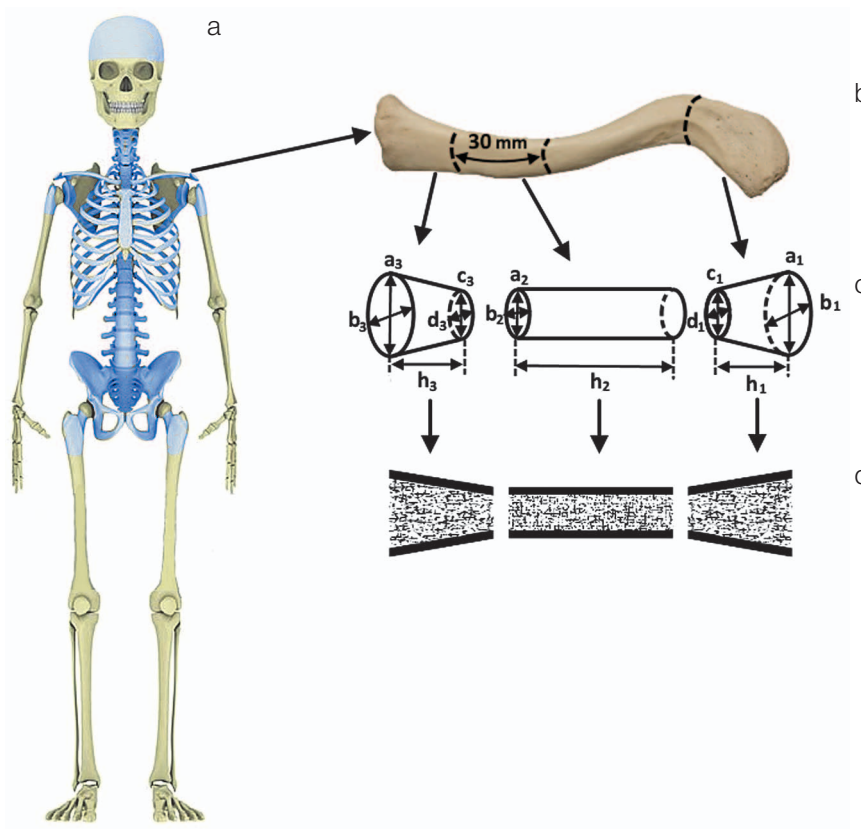


Figure prepared by the authors

Fig. Skeletal regions with active hematopoiesis for a 15-year-old male adolescent and their segmentation illustrated using the clavicle: (a) skeleton of a 15-year-old (regions modeled with active hematopoiesis are highlighted in blue); (b) clavicle (bone segments are outlined by dashed lines); (c) diagram of bone division into basic phantoms of bone segments (BPS) and their linear parameters; (d) BPS of the clavicle in voxel representation, cross section (voxels simulating mineralized bone are shown in black, bone marrow is shown in white)

Table 1. Mass fractions of red bone marrow (RBM) (% of total RBM mass in the skeleton) in the major hematopoietic skeletal sites of 15-year-olds

No.	Hematopoietic site	RBM mass fraction, %	
		15-year-olds	Adults
1	Femur	15.7	5.9
2	Humerus	3.8	3.6
3	Sacrum	8.5	7.4
4	Pelvic bones	18.6	23.2
5	Skull	10.2	6.2
6	Clavicle	1.0	0.8
7	Scapula	3.3	4.7
8	Ribs	13.7	9.8
9	Sternum	1.8	1.8
10	Cervical vertebrae	3.3	3.5
11	Thoracic vertebrae	11.0	17.5
12	Lumbar vertebrae	10.6	15.5

Table prepared by the authors based on data from [19]

Table 2. Bone microarchitecture parameters adopted for the basic phantoms of bone segments of 15-year-old male and female adolescents

Hematopoietic site	BV/TV, %	Tb. Th., mm	Tb. Sp., mm
Femur (neck)	35 (23–43)	0.24 (22)	0.54 (14)
Femur trochanter area	26 (17–32)	0.24 (22)	0.54 (14)
Humerus	22 (11–32)	0.21 (13)	0.58 (32)
Ribs	12 (5–25)	0.14 (12)	0.82 (11)
Ilium	25 (20–29)	0.16 (10)	0.46 (0.07)
Ischium bone and pubic ramus inferior	25 (20–29)	0.16 (10)	0.75 (9)
Skull	52 (41–65)	0.29 (32)	0.57 (35)
Clavicle body	15 (10–20)	0.2 (32)	0.8 (25)
Clavicle ends	29 (15–46)	0.14 (31)	0.8 (25)
Scapula	22 (9–47)	0.24 (42)	0.96 (23)
Sternum	15 (8–22)	0.15 (29)	1.4 (9)
Cervical vertebrae	21 (16–28)	0.14 (14)	0.15 (10)
Thoracic vertebrae	14 (7–19)	0.12 (17)	0.6 (15)
Lumbar vertebrae + Sacrum	14 (7–19)	0.12 (17)	0.6 (15)

Table prepared by the authors based on their own data [25]

Note: parentheses contain the coefficient of variation (CV, %); for BV/TV, the range of possible values is provided; Tb. Th. — trabecular thickness, Tb. Sp. — mean trabecular separation, BV/TV — bone volume fraction.

Table 3. Linear dimensions and cortical bone layer thickness for basic phantoms of bone segments of 15-year-old male and female adolescents

Hematopoietic site	Segment	Shape ¹	Sex	Phantom parameters, mm (parenthetic CV, %) ²					
				<i>h</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>Ct. Th.</i>
Femur	Neck	cyl	m	29.6 (5)	36 (14)	32 (13)	–	–	1.9 (19)
			f	30.5 (5)	29.4 (14)	23.9 (13)	–	–	1.9 (19)
	Trochanter area	dc	m	41 (4)	66 (6)	44 (6)	30 (7)	30 (7)	2.3 (15)
			f	34.5 (5)	58 (7)	39 (7)	27 (6)	27 (6)	2.3 (15)
Humerus	Proximal end	dc	m	28 (12)	56 (5)	56 (5)	19.4 (4)	19.4 (4)	1.1 (16)
			f	24.9 (12)	51.3 (5)	51.3 (5)	19.4 (4)	19.4 (4)	1.1 (16)
Ribs	Ribs ⁴ 1–2	p	m+f	17 (12)	30	7 (14)	–	–	0.7 (38)
	Ribs ⁴ 11–12	p	m+f	11 (18)	30	6 (17)	–	–	0.7 (38)
	Ribs ⁴ 3, 4, 9, 10	p	m+f	13 (8)	30	7 (14)	–	–	1.2 (38)
	Ribs ⁴ 5, 6, 7, 8	p	m+f	14 (14)	30	8 (13)	–	–	0.7 (38)
Sacrum	Body-1	p	m	30 (7)	40 (11)	24.5 (10)	–	–	1.2 (35)
			f	30 (9)	37.8 (11)	22.2 (12)	–	–	1.2 (35)

Table 3 (continued)

Hematopoietic site	Segment	Shape ¹	Sex	Phantom parameters, mm (parentetic CV, %) ²					
				<i>h</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>Ct. Th.</i>
Sacrum	Body-2-3	p	m	46 (8)	28.7 (11)	15 (9)	–	–	1.2 (35)
			f	45.2 (15)	28 (11)	13.8 (13)	–	–	1.2 (35)
	Body-4-5	p	m	36 (9)	28 (11)	8 (13)	–	–	1.2 (35)
			f	35 (14)	28 (11)	8.5 (12)	–	–	1.2 (35)
	Pedicle 1	cyl	m+f	13.9 (14)	23.7 (15)	15.3 (11)	–	–	1.2 (35)
	Pedicle 2	cyl	m+f	14.2 (14)	25 (11)	13.6 (17)	–	–	1.2 (35)
	Pedicle 3	cyl	m+f	13.9 (14)	18.3 (11)	13.2 (14)	–	–	1.2 (35)
	Pedicle 4	cyl	m+f	13.9 (14)	14.5 (11)	11.2 (18)	–	–	1.2 (35)
	Ala 1	p	m	30 (13)	20 (10)	42 (13)	–	–	1.2 (35)
			f	30 (9)	21 (15)	38.6 (8)	–	–	1.2 (35)
	Ala 2	p	m	26 (15)	23 (17)	25 (8)	–	–	1.2 (35)
			f	26 (9)	23 (17)	22.7 (13)	–	–	1.2 (35)
	Ala 3–4	pr	m+f	19 (16)	18 (9)	38.5 (15)	38.5 (15)	–	1.2 (35)
Pelvic bones	Iliac ala	p	m+f	9.5 (31)	30	30	–	–	1 (30)
	Iliac crest	p	m	11 (15)	30	13 (9)	–	–	1 (30)
			f	11 (15)	30 ⁶	13 (9)	–	–	1 (30)
	Iliac dorsal segment	p	m+f	19 (16)	30	30	–	–	1 (30)
	Ischium ramus	cyl	m+f	30 ⁶	34 (9)	25 (8)	–	–	0.5 (30)
	Pubis ramus inferior	dc	m+f	47 (17)	16 (25)	22 (23)	26 (23)	14 (36)	0.5 (30)
	Pubis ramus superior (lower part)	p	m	32 (19)	15 (20)	29 (20)	–	–	0.7 (30) ⁴ 1.5 (12) ⁴
			f	31 (13)	14 (7)	33 (18)	–	–	0.7 (30) ⁴ 1.5 (12) ⁴
	Pubis ramus superior (upper)	p	m	51.2 (8)	14.5 (20)	16 (20)	–	–	0.7 (30) ⁴ 1.5 (12) ⁴
			f	83 (7)	11 (18)	16 (20)	–	–	0.7 (30) ⁴ 1.5 (12) ⁴
	Acetabulum	hc	m+f	29 (10)	26 (10)	21 (20)	–	–	0.5 (30) ⁵ 3.6 (30) ⁵
Skull	Flat bones ⁴	p	m+f	5.2 (12)	30 ⁶	30 ⁶	–	–	1.3 (33) ³ 1.5 (22) ³
Clavicle	Shaft (acromial part)	cyl	m	19.8 (5)	22 (9)	12 (29)	12 (17)	12 (8)	0.8 (26)
			f	20.7 (9)	21 (6)	10 (32)	10 (9)	10 (10)	0.8 (26)
	Body	dc	m	30	12 (17)	12 (8)	–	–	1.8 (2)
			f	30	10 (9)	10 (10)	–	–	1.8 (2)

Table 3 (continued)

Hematopoietic site	Segment	Shape ¹	Sex	Phantom parameters, mm (parenthetic CV, %) ²					
				<i>h</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>Ct. Th.</i>
Clavicle	Shaft (sternal part)	dc	m	19.8 (5)	26 (15)	24 (13)	12 (17)	12 (8)	0.8 (26)
			f	20.7 (9)	24 (16)	21 (14)	10 (9)	10 (10)	0.8 (26)
Scapula	Glenoid	cyl	m+f	16.9 (8)	30.7 (11)	22.3 (18)	–	–	0.9 (28)
	Acromion	p	m+f	8.8 (18)	32.4 (4)	25.2 (4)	–	–	0.8 (13)
	Lateral margin	p	m+f	30	3.5 (3)	10 (12)	–	–	0.8 (13)
Sternum	Body	p	m+f	10.6 (14)	30	30	–	–	0.9 (44)
Cervical vertebrae	Vertebral body 3–7	cyl	m+f	11.9 (11)	15.2 (8)	19 (14)	–	–	0.3 (7)
	Vertebral body 2	p	m+f	19.2 (13)	14.3 (10)	17.5 (3)	–	–	0.3 (7)
	Lateral mass 1	p	m+f	15 (13)	11.4 (9)	10.5 (9)	–	–	0.3 (7)
Thoracic vertebrae	Vertebral body	cyl	m+f	18.6 (16)	28.7 (19)	24.9 (20)	–	–	0.4 (25)
	Lamina+inferior articular. proc.	p	m+f	32 (12)	10.2 (14)	4.2 (13)	–	–	1.3 (16)
	Spinous process	p	m+f	9.3 (18)	33 (18)	4.9 (18)	–	–	0.4 (25)
	Superior articular process	p	m+f	11.4 (12)	11.3 (14)	4.4 (11)	–	–	1.3 (16)
	Transverse process	p	m+f	9.9 (18)	14.8 (18)	8.6 (19)	–	–	0.4 (25)
Lumbar vertebrae	Vertebral body	cyl	m+f	24.7 (12)	31.5 (8)	44.5 (9)	–	–	0.4 (25)
	Lamina+inferior articular. proc.	p	m+f	20.4 (10)	12.7 (13)	4.1 (13)	–	–	1.0 (34)
	Spinous process	p	m+f	24 (10)	31 (10)	5.6 (10)	–	–	0.4 (25)
	Superior articular process	p	m+f	14 (14)	15 (13)	12 (17)	–	–	1.0 (34)
	Transverse process	p	m+f	10.7 (10)	20.5 (11)	5.6 (10)	–	– ⁶	0.4 (25)

Table prepared by the authors based on their own data [25, 36]

Note: m — male; f — female; m+f — BPS was modeled independent of sex; ¹ — phantom shape was designated as follows: cyl — cylinder, dc — deformed cylinder, p — rectangular parallelepiped, pr — prism with triangle base; t — hollow cylinder; ² — BPS dimensions were designated as follows: *h* — height; *a* — major axis (*c*), major axis for a large base (dc) or side *a* (p) or outer diameter (t); *b* — minor axis (*c*), minor axis for a large base (dc) or side *b* (p) or inner diameter (t); *c* — major axis for a small base (dc); *d* — minor axis for a small base (dc); for prism(pr): *a*, *b*, *c* — sides of the triangle base; ³ — cortical layer thickness was assumed to be different for the inner (medial) and outer (gluteal) surfaces of a given segment of the skull; ⁴ — symphyseal surface covered by thicker cortical layer than other surfaces of BPS; ⁵ — a higher *Ct. Th.* value assumed for the medial side of the acetabulum; ⁶ — BPS imitated only a part of the simulated bone segment, when the bone segment dimensions exceeded significantly 30 mm, since in terms of dosimetry in such cases it makes no sense to simulate the entire bone fragment; “–” — dimension is not used for constructing the phantom of this segment.

adolescents are presented in Table 3. The data sources used to obtain these parameters are presented in [24].

Unlike bone microstructural parameters, the linear dimensions of several bones depend on sex; therefore, their dimensions were estimated separately for males and females. The SPSPD phantoms of the skeleton for 15-year-old male and female adolescents consist of

46 BPSs, of which 14 segments are specific to males, another 14 are specific to females, and 32 segments were modeled identically for both sexes, as shown in Table 3. The sacrum was the most segmented skeletal region with active hematopoiesis. It comprised 10 BPSs, while a single BPS was used to model both the humerus and the skull.

The basic phantoms of bone segments (BPS) for a 15-year-old adolescent are mostly shaped as cylinders or rectangular parallelepipeds. Their dimensions ranged 3.5–66 mm. Sexual dimorphism is characteristic of many BPSs, with the greatest differences observed in the inferior part of the superior pubic ramus, at the level of 66%. The cortical bone layer of the modeled phantoms ranged from 0.3 mm (cervical vertebral bodies) to 2.3 mm (proximal end of the femur). *BV/TV* varied 12–52%, trabecular thickness ranged 0.1–0.29 mm, and the trabecular separation was 0.45–1.4 mm (Table 4).

The population variability of individual linear phantom dimensions ranged from 3% (cervical vertebral bodies) to 36% (inferior pubic ramus), with an average of 30% across all dimensions of all BPSs. The greatest variability in the cortical bone layer thickness was estimated for the sternum — 44%, while the smallest was for the cervical vertebral bodies — 3%. On average, the variability of *Ct.Th.* was 27%. For microarchitecture parameters, this indicator ranged from 6% to 42%. The volume of the generated SPSs ranged 23–264% of the BPS volume.

DISCUSSION

We used approximately 100 literature sources describing bone samples (> 5000) from 4592 individuals [24] to estimate the parameters of phantoms for 15-year-old male and female adolescents. A total of 197 parameters characterizing linear dimensions and 138 parameters characterizing the microstructure of the skeletal

bones were evaluated to construct these phantoms. As mentioned earlier, some parameters for the phantoms of 15-year-old male and female adolescents were set equal to those for adults, specifically microarchitecture parameters for the clavicle, ribs, scapula, skull, cervical vertebrae, and sternum, as well as linear dimensions for the femoral neck, humerus, pelvic bones, and ribs.

By the age of 15, ossification processes are complete in many skeletal regions, and these regions were modeled using the same set of segments as for adults, although with different parameters. These include the femur, humerus, pelvic bones, sacrum, skull, ribs, and scapula. On the other hand, some skeletal regions modeled for adults were not modeled for 15-year-olds, such as the articular processes and arches of the thoracic and lumbar vertebrae. Consequently, the skeletal phantom for 15-year-old male and female adolescents consists of a smaller number of segments than adult phantoms, but a greater number than phantoms for younger children.

The RBM distribution in 15-year-olds is similar to that in adults; however, the RBM fraction in the vertebrae is greater in adults than in 15-year-olds, while the RBM fraction in the femur and humerus is, conversely, smaller.

The *BV/TV* for the BPS of 15-year-olds averages 20%, which is slightly higher than that for adults (17.3%). Age-related changes in *Tb. Th.* are insignificant, whereas *Tb. Sp.* for 15-year-olds is 18% lower than for adults. Table 4 presents a comparison of the media volumes within the BPS for 15-year-old male and female adolescents, as well as for adult males and females.

Table 4. Comparison of the basic phantoms of bone segment volumes for a 15-year-old child and adult men and women

BPS	Modeled media	Volume of modeled structure, cm ³			
		Adults		15-year-old child	
		Male	Female	Male	Female
Femur neck	BM	18.43	10.39	13.6	7.92
	TB	3.68	2.14	7.38	4.31
	CB	5.94	4.58	5.8	4.62
	Entire BPS	28.05	17.11	25.35	16.85
Proximal humerus	BM	32.26	24.2	23.9	18.36
	TB	2.02	1.54	6.27	4.82
	CB	3.47	3.14	3.54	2.95
	Entire BPS	37.75	28.88	33.71	26.13
First sacral vertebra (S1) body	BM	21.87	18.37	23.07	19.53
	TB	3.84	3.24	3.55	3.1
	CB	3.70	3.47	2.79	2.55
	Entire BPS	29.41	25.08	29.41	25.18

Table prepared by the authors based on their own data [25, 36]
Note: BM — bone marrow; CB — cortical bone; TB — trabecular bone; BPS — basic phantoms of bone segments.

Due to their smaller linear dimensions, the total volume of the BPS for 15-year-old adolescents is 21% less than that for adults. The sexual dimorphism in the average BPS volumes was 11%, and for individual segments, it reached 73% (ribs 11–12).

CONCLUSION

The present work is the sixth in a series of articles devoted to the parameters of computational skeletal phantoms for humans of different ages and sexes. In this article, we provide a description of SPSPD phantoms of the skeleton for 15-year-old male and female adolescents. The phantoms consist of 46 BPSs. For 14 out of the 46 segments,

sexual dimorphism in linear dimensions was taken into account.

Based on the assessment of parameter variability, 12 SPSs were generated for each BPS. The DF calculated for the SPSs will be used to assess their population variability.

The generated phantoms can be applied for the internal dosimetry of bone-seeking beta-emitters in the population, as well as for the dosimetry of other beta-emitting radionuclides, including those used in radionuclide therapy, such as ^{89}Sr , ^{32}P , ^{186}Re , ^{188}Re , and $^{117\text{m}}\text{Sn}$.

Further research will focus on DF calculating, as well as on developing computational phantoms for the internal dosimetry of bone-seeking radionuclides during the intrauterine period.

References

- Krestinina LY, Epifanova S, Silkin S, Mikryukova L, Degteva M, Shagina N, et al. Chronic low-dose exposure in the Techa River Cohort: risk of mortality from circulatory diseases. *Radiation and Environmental Biophysics*. 2013;52(1):47–57. <https://doi.org/10.1007/s00411-012-0438-5>
- Akleyev AV. *Chronic radiation syndrome among residents of the Techa River riverside villages*. Chelyabinsk: Book; 2012 (In Russ.).
- Preston DL, Sokolnikov ME, Krestinina LY, Stram DO. Estimates of Radiation Effects on Cancer Risks in the Mayak Worker, Techa River and Atomic Bomb Survivor Studies. *Radiation Protection Dosimetry*. 2017;173(1–3):26–31. <https://doi.org/10.1093/rpd/ncw316>
- Spiers FW, Beddoe AH, Whitwell JR. Mean skeletal dose factors for beta-particle emitters in human bone. Part I: volume-seeking radionuclides. *The British Journal of Radiology*. 1978;51(608):622–7. <https://doi.org/10.1259/0007-1285-51-608-622>
- Degteva MO, Napier BA, Tolstykh EI, Shishkina EA, Shagina NB, Volchkova AY, et al. Enhancements in the Techa River Dosimetry System: TRDS-2016D Code for Reconstruction of Deterministic Estimates of Dose from Environmental Exposures. *Health Physics*. 2019;117(4):378–87. <https://doi.org/10.1097/HP.0000000000001067>
- O'Reilly SE, DeWeese LS, Maynard MR, Rajon DA, Wayson MB, Marshall EL, et al. An image-based skeletal dosimetry model for the ICRP reference adult female–internal electron sources. *Physics in Medicine and Biology*. 2016;61(24):8794–824. <https://doi.org/10.1088/1361-6560/61/24/8794>
- Xu XG, Chao TC, Bozkurt A. VIP-Man: an image-based whole-body adult male model constructed from color photographs of the Visible Human Project for multi-particle Monte Carlo calculations. *Health Physics*. 2000;78(5):476–86. <https://doi.org/10.1097/00004032-200005000-00003>
- Shah AP, Bolch WE, Rajon DA, Patton PW, Jokisch DW. A paired-image radiation transport model for skeletal dosimetry. *Journal of Nuclear Medicine*. 2005;46(2):344–53.
- Hough M, Johnson P, Rajon D, Jokisch D, Lee C, Bolch W. An image-based skeletal dosimetry model for the ICRP reference adult male–internal electron sources. *Physics in Medicine and Biology*. 2011;56(8):2309–46. <https://doi.org/10.1088/0031-9155/56/8/001>
- Bolch WE, Eckerman K, Endo A, Hunt JGS, Jokisch DW, Kim CH, et al. ICRP Publication 143: Paediatric Reference Computational Phantoms. *Annals of the ICRP*. 2020;49(1):5–297.
- Degteva MO, Tolstykh EI, Shishkina EA, Sharagin PA, Zalyapin VI, Volchkova AY, et al. Stochastic parametric skeletal dosimetry model for humans: General approach and application to active marrow exposure from bone-seeking beta-particle emitters. *PLOS One*. 2021;16(10):e0257605. <https://doi.org/10.1371/journal.pone.0257605>
- Sharagin PA, Shishkina EA, Tolstykh EI. Computational red bone marrow dosimetry phantom of a one-year-old child enabling assessment of exposure due to incorporated beta emitters. *Extreme Medicine*. 2023;24(4):74–82 (In Russ.). <https://doi.org/10.47183/mes.2022.045>
- Sharagin PA, Shishkina EA, Tolstykh EI. Computational red bone marrow dosimetry phantom of a one-year-old child enabling assessment of exposure due to incorporated beta emitters. *Extreme Medicine*. 2023;25(3):44–55 (In Russ.). <https://doi.org/10.47183/mes.2023.030>
- Sharagin PA, Tolstykh EI, Shishkina EA. Computational phantom for a 5-year old child red bone marrow dosimetry due to incorporated beta emitters. *Extreme Medicine*. 2023;25(4):86–97 (In Russ.). <https://doi.org/10.47183/mes.2023.061>
- Sharagin PA, Tolstykh EI, Shishkina EA. Computational phantom for the dosimetry of the red bone marrow of a 10-year-old child due to incorporated beta-emitters. *Extreme Medicine*. 2024;26(2):38–48 (In Russ.). <https://doi.org/10.47183/mes.2024.032>
- Sharagin PA, Tolstykh EI, Shishkina EA. Computational phantom for red bone marrow dosimetry in adult males and females. *Extreme Medicine*. 2025;27(2):220–8 (In Russ.). <https://doi.org/10.47183/mes.2025-286>
- Cristy M. Active bone marrow distribution as a function of age in humans. *Physics in Medicine and Biology*. 1981;26(3):389–400. <https://doi.org/10.1088/0031-9155/26/3/003>
- Robinson RA. Chemical analysis and electron microscopy of bone. *Bone as a tissue*. New York: McGraw-Hill; 1960.
- Vogler JB. 3rd, Murphy WA. Bone marrow imaging. *Radiology*. 1988;168(3):679–93. <https://doi.org/10.1148/radiology.168.3.3043546>
- Vande Berg BC, Malghem J, Lecouvet FE, Maldague B. Magnetic resonance imaging of the normal bone marrow. *Skeletal Radiology*. 1998;27:471–83. <https://doi.org/10.1007/s002560050423>
- Vande Berg BC, Malghem J, Lecouvet FE, Maldague B. Magnetic resonance imaging of normal bone marrow. *European Radiology*. 1998;8(8):1327–34. <https://doi.org/10.1007/s003300050547>
- Taccone A, Oddone M, Dell'Acqua AD, Occhi M, Ciccone MA. MRI “road-map” of normal age-related bone marrow.

- II. Thorax, pelvis and extremities. *Pediatric Radiology*. 1995;25(8):596–606.
<https://doi.org/10.1007/bf02011826>
23. Taccone A, Oddone M, Occhi M, Dell'Acqua AD, Ciccone MA. MRI «road-map» of normal age-related bone marrow. I. Cranial bone and spine. *Pediatric Radiology*. 1995;25(8):588–95.
<https://doi.org/10.1007/bf02011825>
24. Tolstykh EI, Sharagin PA, Shishkina EA, Volchkova AYU, Smith MA, Napier BA. Stochastic parametric skeletal dosimetry model for human: anatomical-morphological basis and parameter evaluation. *PLOS One*. 2025;20(7):e0327156.
<https://doi.org/10.1371/journal.pone.0327156>
25. Sharagin PA, Shishkina EA, Tolstykh EI, Volchkova AYU, Smith MA, Degteva MO. Segmentation of hematopoietic sites of human skeleton for calculations of dose to active marrow exposed to bone-seeking radionuclides. *Conference proceedings of sixth international conference on radiation and applications in various fields of research*. Macedonia; 2018.
<https://doi.org/10.21175/RadProc.2018.33>
26. Shishkina EA, Sharagin PA, Volchkova AYU. Analytical Description of the Dose Formation in Bone Marrow due to ⁹⁰Sr Incorporated in Calcified Tissues. *Radiation Safety Issues*. 2021;3:72–82 (In Russ.).
EDN: [LYGTKD](https://doi.org/10.1016/S0146-6453(03)00002-2)
27. Valentin J. Basic anatomical and physiological data for use in radiological protection: reference values. *Annals of the ICRP*. 2002;32(3–4):1–277.
[https://doi.org/10.1016/S0146-6453\(03\)00002-2](https://doi.org/10.1016/S0146-6453(03)00002-2)
28. Woodard HQ, White DR. The composition of body tissues. *British Journal of Radiology*. 1986;59:1209–18.
<https://doi.org/10.1259/0007-1285-59-708-1209>
29. Shishkina EA, Timofeev YS, Volchkova AY, Sharagin PA, Zalyapin VI, Degteva MO, et al. Trabecula: A Random Generator of Computational Phantoms for Bone Marrow Dosimetry. *Health Physics*. 2020;118(1):53–9.
<https://doi.org/10.1097/hp.0000000000001127>
30. Parisien MV, McMahon D, Pushparaj N, Dempster DW. Trabecular architecture in iliac crest bone biopsies: intra-individual variability in structural parameters and changes with age. *Bone*. 1988;9(5):289–95.
[https://doi.org/10.1016/8756-3282\(88\)90012-9](https://doi.org/10.1016/8756-3282(88)90012-9)
31. Hazrati Marangalou J, Ito K, Taddei F, van Rietbergen B. Inter-individual variability of bone density and morphology distribution in the proximal femur and T12 vertebra. *Bone*. 2014;60:213–20.
<https://doi.org/10.1016/j.bone.2013.12.019>
32. Van Dessel J, Huang Y, Depypere M, Rubira-Bullen I, Maes F, Jacobs R. A comparative evaluation of cone beam CT and micro-CT on trabecular bone structures in the human mandible. *Dento Maxillo Facial Radiology*. 2013;42(8):20130145.
<https://doi.org/10.1259/dmfr.20130145>
33. Fanuscu MI, Chang TL. Three-dimensional morphometric analysis of human cadaver bone: microstructural data from maxilla and mandible. *Clinical Oral Implants Research*. 2004;15(2):213–8.
<https://doi.org/10.1111/j.1600-0501.2004.00969.x>
34. Ibrahim N, Parsa A, Hassan B, van der Stelt P, Aartman IH, Nambiar P. Influence of object location in different FOVs on trabecular bone microstructure measurements of human mandible: a cone beam CT study. *Dento Maxillo Facial Radiology*. 2014;43(2):20130329.
<https://doi.org/10.1259/dmfr.20130329>
35. Shishkina EA, Sharagin PA, Tolstykh EI, Smith MA, Napier BA, Degteva MO. Uncertainty of stochastic parametric approach to bone marrow dosimetry of ^{89,90}Sr. *Heliyon*. 2024;10(4):e26275.
<https://doi.org/10.1016/j.heliyon.2024.e26275>
36. Sharagin PA, Shishkina EA, Tolstykh EI, Smith MA, Napier BA. Stochastic parametric skeletal dosimetry model for humans: Pediatric and adult computational skeleton phantoms for internal bone marrow dosimetry. *PLOS One*. 2025;20(7):e0327479.
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IMPACT OF ICE-WATER SWIMS ON CARDIAC CONDUCTION AND AUTONOMIC REGULATION IN WINTER SWIMMERS

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Introduction. As a kind of sport, winter swimming differs from pool- or open-water swimming due to the extreme cold stress it places on the body. Swims in cold water with facial immersion trigger a diving reflex, which may be accompanied by the development of cardiac arrhythmias under low-temperature conditions. We believe that specific criteria should be developed for permitting individuals to engage in cold-water swimming.

Objective. Study of the characteristics of changes in the state of the cardiovascular and nervous systems, autonomic regulation, and metabolic parameters in winter swimmers at water temperatures from +0.5 °C to +2.0 °C both over various standard distances and during a 10-min swim of 450–550 m.

Materials and methods. Under competitive conditions and during training camps (at water temperatures from +0.5 °C to +1.5 °C and air temperatures from –15 °C to –18 °C), 24 winter swimmers were examined. These were 9 women aged 23–55 (mean age 39.1 ± 2.7 years); 3 men aged 71 ± 3 years; and 12 men aged 35–60 (mean age 43 ± 1.8 years), all of whom had received medical clearance for participation. The participants' condition was analyzed before and after swims at distances of 25 m backstroke, 25 m and 200 m front crawl (freestyle), as well as after a 10-min swim of 400–450 m. The functional state of the body was assessed using 12-lead electrocardiography, blood pressure measurement, determination of capillary blood glucose concentration, and assessment of simple sensorimotor reaction to a light stimulus. Statistical analysis to identify differences between parameters was performed using the GraphPad Prism 8 software package for Windows 10.

Results. A statistically significant increase in the mean capillary blood glucose levels was observed after swims of 25 m ($p < 0.05$), 200 m ($p < 0.01$), and 400 m compared to the baseline. An increase in the simple sensorimotor reaction time after 10-min swims was noted; thus, the baseline of 263 ± 10 ms vs. 328 ± 21 ms after the swim ($p < 0.01$). According to ECG data, at baseline, 67% of the examined individuals showed a widened P wave > 0.11 ms. After short-distance swims of 25 m, P wave duration exceeded the upper limit of normal in 85% of the examined. Compared to backstroke swims, swims with facial immersion in water were accompanied by a significantly more pronounced widening of the P wave ($p < 0.05$), slowing of atrial conduction PQ ($p < 0.01$), and a more pronounced increase in the QTc interval ($p < 0.05$). The QTc value progressively increased in accordance with the duration of the swims. During a 10-min swim, 50% of the examined individuals showed a QTc > 500 ms.

Conclusions. Low water temperature is a factor that, even in cold-adapted athletes, provokes stress accompanied by an increase in blood glucose. During swims in cold water with facial immersion, the activated diving reflex triggers cardiac arrhythmias. During long-distance swims, conduction in the nervous system slows down, manifested in an increased simple sensorimotor reaction time and slowed myocardial conduction, reaching pathological values. In this regard, we believe that an additional criterion for medical clearance — assessment of the response of the cardiovascular system to cold-water immersion — should be developed and implemented for beginners wishing to engage in cold-water swimming, in order to avoid the risk of a pathological cardiovascular reaction.

Keywords: cold-water swimming; nervous system; cardiovascular system; autonomic regulation; cardiac arrhythmias; intracardiac conduction; hypertensive response; sensorimotor reaction; cold-water immersion

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ВЛИЯНИЕ ЗАПЛЫВОВ В ЛЕДЯНОЙ ВОДЕ НА ПРОВОДЯЩУЮ СИСТЕМУ СЕРДЦА И ВЕГЕТАТИВНУЮ РЕГУЛЯЦИЮ У ПЛОВЦОВ ЗИМНЕГО ПЛАВАНИЯ

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Введение. Зимнее плавание как вид спорта отличается от классического плавания и плавания в открытой воде экстремальной холодовой нагрузкой на организм. При заплывах в холодной воде с погружением лица в воду реализуется нырательная реакция, которая в условиях низких температур может сопровождаться формированием сердечных аритмий. Мы полагаем, что для допуска к занятиям холодным плаванием необходима разработка специальных критериев.

Цель. Изучение особенностей изменений состояния сердечно-сосудистой, нервной систем, вегетативной регуляции и метаболических показателей у пловцов зимнего плавания при температуре воды от +0,5 до +2,0 °C на различных дистанциях и 10-минутном заплыве на 450–550 м.

Материалы и методы. В условиях соревновательной деятельности и учебно-тренировочных сборов (при температуре воды от +0,5 до +1,5 °C, температуре воздуха от -15 до -18 °C) проведено обследование 24 спортсменов зимнего плавания: 9 женщин (23–55 лет; средний возраст 39,1 ± 2,7 года); 3 мужчин в возрасте 71 ± 3 года; 12 мужчин (35–60 лет; средний возраст 43,0 ± 1,8 года), имевших медицинский допуск к занятиям. Состояние испытуемых анализировали до и после заплывов на дистанциях 25 м кролем на спине, 25 и 200 м вольным стилем на груди и после 10-минутного заплыва на 400–450 м. Для оценки функционального состояния проведены: электрокардиография в 12 отведениях, измерение артериального давления, определение концентрации глюкозы в капиллярной крови, определение простой сенсомоторной реакции на световой раздражитель. Статистический анализ для выявления различий между показателями выполнен с использованием пакета программного обеспечения GraphPad Prism 8 для Windows 10.

Результаты. Установлено статистически значимое повышение среднегрупповых показателей уровня глюкозы в капиллярной крови после заплывов на 25 м ($p < 0,05$), 200 м ($p < 0,01$) и 400 м относительно исходного состояния; увеличение времени простой сенсомоторной реакции после 10-минутных заплывов: в исходном состоянии 263 ± 10 и 328 ± 21 мс после заплыва ($p < 0,01$). По данным ЭКГ в исходном состоянии у 67% обследованных выявлено уширение зубца Р $> 0,11$ мс. После заплывов на короткие дистанции 25 м длительность зубца Р выходит за верхний предел нормы у 85% обследованных. По сравнению с заплывами на спине заплывы с погружением лица в воду сопровождались достоверно более выраженным уширением зубца Р ($p < 0,05$), замедлением проведения через предсердия — PQ ($p < 0,01$), более выраженным увеличением QTc показателя ($p < 0,05$). Величина QTc прогрессивно увеличивается в соответствии с длительностью заплывов. При 10-минутном заплыве у 50% обследованных QTc > 500 мс.

Выводы. Низкая температура воды — фактор, который даже у адаптированных к холоду спортсменов провоцирует стресс, сопровождающийся повышением глюкозы в крови. При заплывах с погружением лица в воду реализуется нырательный рефлекс, который в холодной воде может провоцировать сердечные аритмии. При заплывах на длинные дистанции происходит замедление проведения в нервной системе, что отражается в увеличении времени простой сенсомоторной реакции и замедлении проводимости миокарда, достигающего патологического значения. В связи с этим считаем, что для новичков, желающих заниматься холодным плаванием, во избежание риска патологического ответа сердечно-сосудистой системы необходима разработка и введение в практику допуска дополнительного критерия — оценки реакции сердечно-сосудистой системы на водно-холодовую иммерсию.

Ключевые слова: холодное плавание; нервная система; сердечно-сосудистая система; автономная регуляция; сердечные аритмии; внутрисердечное проведение; гипертензивный ответ; сенсомоторная реакция; водно-холодовая иммерсия

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Соответствие принципам этики: исследование проведено в соответствии с Хельсинкской декларацией и одобрено Этическим комитетом Санкт-Петербургского государственного университета (протокол № 40 от 07.03.2012). Все испытуемые предварительно были ознакомлены с протоколом обследования, подписали согласие об участии в нем и дали разрешение на публикацию обезличенных данных.

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INTRODUCTION

Today, cold-water (also referred to as winter or ice) swimming has gained popularity worldwide. In 2022, winter swimming was included in the All-Russian Register of Sports in the Russian Federation.¹ Despite a relatively well-developed system of amateur winter swimming, there is a lack of systematic scientific research in the field of training winter swimming athletes. Therefore, qualified scientific research is required to address various related problems in this field. Among the most important of these are the categorization of competition distances and determining their sequence during competitions. The classification of winter swimming competition categories used in the Russian Federation is based on the categories proposed by the International Winter Swimming Association (IWSA),² where category “A” (ice water) with a water temperature from 0°C to +2°C is intended for distances of 25/50/100/200 m; category “B” (freezing water) with a water temperature from +2.1°C to +5°C — for distances of 25/50/100/200/400 m; category “C” (cold water) with a water temperature from +5.1°C to +9°C — for distances of 25/50/100/200/400/500/1000 m.

During competitions in cold water, several extreme factors act on the athlete's body. First, competitive stress is accompanied by activation of the sympathoadrenal system [1], mobilizing the body's functional reserves. Second, the exposure to cold water triggers a set of protective compensatory reactions against hypothermia, such as reflex constriction of peripheral vessels, redistributing blood toward internal organs (the “core”), and activation of heat production responses (shivering and non-shivering thermogenesis). Third, immersion in cold water with the head triggers a set of cardiovascular reactions, referred to as the diving reflex, consisting in the following effects. Under the influence of adrenergic effects, sharp vasoconstriction of peripheral vessels occurs. Simultaneously, the increased cholinergic influence on the myocardium leads to a reflex slowing of the heart rate. At the same time, stimulation of the respiratory tract causes reflex apnea and bronchiolar constriction; as a result, vessels in the pulmonary artery, cerebral, and coronary circulation dilate, redistributing blood toward these areas [1, 2].

Simultaneous activation of parasympathetic (diving reflex) and sympathetic (cold stress, competitive conditions) effects on the heart can lead to the so-called “autonomic conflict,” which, during water immersion at low temperatures, may result in the formation of arrhythmias, including those of a fatal nature [3]. It should also be noted that physical and cold-related loads stimulate an increase in oxidative processes followed by ATP production and heat release, which is intended to maintain body temperature. Inclusively, all this raises the load on the body's gas transport systems and, above all, on the

cardiovascular system. However, even a slight drop in body temperature in an aggressively cold environment by fractions of a degree can disrupt nervous system functions and slow intracardiac conduction, while a powerful peripheral vasospasm can lead to increased blood pressure.

In this work, we aim to study the characteristics of changes in the state of the cardiovascular and nervous systems, autonomic regulation, and metabolic parameters in winter swimmers at water temperatures from +0.5°C to +2.0°C over various distances and during a 10-min swim of 450–550 m. To that end, the following objectives are set:

(1) to assess myocardial conduction (duration of P, PQ, QRS, QT, QTc waves, heart axis) in cold-adapted winter swimmers at rest and its changes under the influence of swims of varying duration (25 m backstroke, 25 m and 200 m freestyle, and a 10-min swim of 400–450 m) at water temperatures from +0.5°C to +1.5°C;

(2) to investigate the influence of the diving reflex on myocardial conduction during cold-water swims by comparing 25 m backstroke and freestyle swims;

(3) to study changes in systemic hemodynamics (systolic and diastolic blood pressure, heart rate) during short-term and prolonged cold exposure;

(4) to assess changes in capillary blood glucose levels and simple sensorimotor reaction time as integral parameters of the stress-response and neuromuscular reaction to cold-water immersion.

MATERIALS AND METHODS

The study was conducted under competitive conditions and during training camps held in Pereslavl-Zalessky on January 12–14, 2024 (Friendship Cup) and in Moscow on February 23–24, 2024 (Friendship Cup). All participants were considered conditionally healthy, received medical clearance for competitions, were adapted to cold-water swimming, had at least two years of experience in winter swimming, and had completed the necessary cold-load training.

According to the criteria of the International Ice Swimming Association (IISA), the athletes were permitted to swim specific distances given the presence of a confirmation of swimming the corresponding distance in category “A” during their training process. The functional state of the participants was assessed after swims over distances of 25 m backstroke, 25 m and 200 m freestyle. Additionally, outside of competitions, a 10-min swim over a distance of 400 m was conducted. This distance, although complying with IISA requirements, is not currently regulated by the rules of winter swimming in the Russian Federation. Discussions are currently underway regarding the need to include this distance, which prompted us to conduct this study.

¹ Order of the Ministry of Sports of the Russian Federation No. 333 «On Recognition and Inclusion of Sports Disciplines and a Sport in the All-Russian Register of Sports and on Amendments to the All-Russian Register of Sports» dated 12.04.2022.

² The International Winter Swimming Association. General rules. <https://iwsa.me/winter-swimming-world-championship/rules/>

In total, 24 individuals were examined. These were 9 females (aged 23–55, mean age 39.1 ± 2.7 years); 3 males (aged 71 ± 3 years); and 12 male (aged 35–60, mean age 43 ± 1.8 years) athletes. Swims were conducted in the morning. Water temperature ranged from $+0.5^\circ\text{C}$ to $+1.5^\circ\text{C}$ under an air temperature from -15°C to -18°C . In accordance with the rules of the International Winter Swimming Association (IWSA and IISA), participants swam without wetsuits. For short-distance swims, athletes (men and women aged 23–39) with clearance for swimming and at least two years of cold-water swimming experience were randomly selected.

For the 350–450 m (10-min swim) group, 10 individuals — 9 men and 1 woman aged 35–55 (mean age 43 ± 2.3 years) — were selected. All had clearance for long-distance swims according to the IISA rules and the Winter Swimming Rules approved by the Russian Ministry of Sports.³ Almost all athletes participating in the training camps and competitions who had clearance for the 400 m distance (10-min swim) were examined. These same swimmers were included in the groups for post-swim assessment after the 25 m and 200 m distances. According to the analysis of protocols, the swim times for 25 m ranged 12.4–32.0 s; the control time for 200 m swims was 5 min for men and 5 min 30 s for women.

Beginners in their first season were not included in the groups to exclude insufficiently adapted individuals to cold stress during category “A” swims. The groups were formed such that to proportionally match by age the composition of those engaged in cold-water swimming within the All-Russian Winter Swimming Federation.

The baseline assessment of the body’s functional state was conducted at 8:00–9:00 a.m. before food intake. To that end, methods available under competitive conditions were used: 12-lead electrocardiography (ECG) (recording duration 10 s, recording speed 50 mm/s) using a Polyspectrum-8/E cardioanalyzer and a Neurosoft electrocardiograph (Russia). The presence of arrhythmias and myocardial conduction were assessed: the duration of cardiac cycle intervals (in ms) — R-R, P, PQ, QRS, QT; the QTc index was determined using Bazett’s formula; the electrical axis of the heart (in degrees) was determined. To identify risks of a hypertensive response, blood pressure levels (BP in mmHg) were measured (A&D Medical UA-787 tonometer, Japan). The risk of a hyperglycemic response to cold-water swims was assessed by measuring capillary blood glucose levels (mmol/L) using a One Touch Select Plus device (LifeScan Europe GmbH, China).

The influence of water-cold immersion on the functional state of the nervous system and the speed of neuromuscular conduction was assessed by the time of a simple sensorimotor reaction (SSMR in ms) to a light stimulus (12 responses were recorded) using an

UPFT-1/30 “Psychophysilogist” device (Medicom-MTD, Russia).

Anthropometric parameters of the swimmers were measured: height (in cm) using a wall-mounted medical height rod (tape measure) (KaWe, Germany) and body weight (in kg) using portable medical scales (VMEN-150-50/100-D2-A). The body mass index was calculated using the Quetelet formula, i.e., $\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$.⁴ Functional parameters were recorded both before the swim (15 min prior) and immediately after the swim during recovery. Post-swim ECG, as well as blood pressure (BP) levels, were recorded at 5–7 min of recovery. Capillary blood glucose levels were determined within 7–10 min after the swim; assessment of the simple sensorimotor reaction to a light stimulus was conducted within 10–12 min after the swim.

During data analysis, pre- and post-swim parameters were compared for all distances, and parameters after all swims were also compared. For statistical data analysis, the GraphPad Prism 8 package for Windows 10 was used. The significance of differences for unpaired independent sample pairs was assessed using non-parametric Mann–Whitney U and Kruskal–Wallis tests. For paired related samples (“pre- and post-” swim), the Wilcoxon signed-rank test was used. In samples with normal distribution, the mean and standard error of the mean ($M \pm m$) were calculated for the groups as a whole. To assess the significance of differences, Student’s t -test and one-way ANOVA were used. The level of p -values < 0.05 was considered statistically significant. Additionally, for SSMR and ECG parameters, the statistical significance of changes was determined individually for each subject both pre- and post-swim. To assess the statistical significance of individual parameter changes, the nonparametric Wilcoxon signed-rank test was used.

RESULTS

Anthropometric parameters of amateur athletes adapted to cold-water swimming

The average height of women was 167.1 ± 1.9 cm (155–175 cm); weight — 64.4 ± 2.1 kg (53–72 kg); body mass index (BMI) — 22.7 ± 2.4 kg/m² (20.4–25.2 kg/m²). The average height of men in the main group was 178.8 ± 4.1 cm (169–190 cm); weight — 80.9 kg (63–107 kg); BMI — 25.3 ± 4.5 kg/m² (20.1–34.3 kg/m²). The height of the older men was 189 cm, 181 cm, 182 cm; weight — 103 kg, 84 kg, 89 kg respectively; BMI — 31.1 kg/m², 23.5 kg/m², 24.4 kg/m². The body mass index of the swimmers did not exceed normal values and averaged 22.7 ± 2.4 kg/m² (20.4–25.1 kg/m²) for the group; one woman was found to be underweight (BMI = 17.9 kg/m²). Among male winter swimmers, three individuals were identified with overweight and a BMI of 25.5–29.8 kg/m², and two individuals with body weight

³ Order of the Ministry of Sports of the Russian Federation No. 1026 «On Approval of the Rules for the Sport ‘Winter Swimming’» dated 16.10.2024.

⁴ Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. *Nutrition Today*. 2015;50(3):117–28. <https://doi.org/10.1097/nt.0000000000000092>

corresponding to first-degree obesity (BMI = 31.2 kg/m² and 34.5 kg/m²).

It should be noted that all men with excess weight had been adapted to long-distance swims of 400 m and prolonged exposure to ice-cold water. The other five swimmers included in the 10-min swim group (350–450 m) had a body mass index close to the upper limit of the WHO normal range.⁵

Changes in myocardial conduction under the influence of swims of varying duration in open cold water

The analysis of baseline ECG, recorded at rest in athletes adapted to cold-water swimming, revealed a slowed conduction through the atria in 16 out of 24 individuals (P wave duration exceeded the norm >110–138 ms). In 4 athletes, slowed excitation propagation through the ventricles was observed (QRS 102–116 ms); and in 6 individuals, QTc exceeded the normal range (452–466 ms) (Table 1).

Swims over the distances were performed using the following swimming techniques: backstroke without facial immersion in water and freestyle, i.e., front crawl with

facial immersion in water. More pronounced changes in myocardial conduction were identified during 25 m free-style swims with facial immersion. On average across the group, a statistically significant slowing of impulse conduction velocity through the atria (increase in P wave duration) and from the sinus node to the ventricles (increase in PQ interval) was observed (Table 1). In 3 individuals, P wave duration significantly exceeded the norm (>120 ms) after the swim. The PQ interval significantly exceeded normal limits in 2 individuals (>210 ms). Overall, across the group, an increase in the duration of excitation propagation through the ventricles was observed in the form of a statistically significant prolongation of the QRS interval ($p < 0.05$); in 7 out of 17 individuals, this parameter significantly exceeded normal limits and surpassed 100 ms. A significant ($p < 0.001$) increase in the corrected QTc interval was also noted in 8 out of 17 swimmers, reaching 485 ms or more, which significantly exceeded normal values.

The most pronounced changes in myocardial excitation conduction were identified after long swims of 200 m and 400–450 m over 10 min. For instance, the duration of R–R intervals decreased statistically significantly after the 10-min swim in almost all athletes. In this setting, a

Table 1. Dynamics of myocardial conduction during swims of varying duration in cold water ($t = +0.5^{\circ}\text{C}$)

Electrocardiogram parameters							
Heart rate, bpm	RR mean, ms	P, ms	PQ, ms	QRS, ms	QT, ms	QT, s	QRS axis, degrees
Baseline values, $n = 24$							
75.5 ± 2.4 (57–97)	810 ± 37 (620–1090)	112 ± 3 (90–138)	154 ± 3 (138–201)	93 ± 3 (108–116)	382 ± 7 (354–451)	425 ± 5 (395–466)	49 ± 9 (–24–95)
Swim 25 m backstroke (swim duration range 17–32 s), $n = 13$							
68.4 ± 3.9 (55–95)	906 ± 60 (718–1100)	115 ± 7 (80–148)	160 ± 5 (140–187)	98 ± 4 (84–112)	409 ± 13 (398–473)	434 ± 10 (409–455)	41 ± 14 (–32–96)
Swim 25 m freestyle (swim duration range 15–31 s), $n = 17$							
79.5 ± 3.7 (54–103) [#]	761 ± 35 (584–1106) [#]	139 ± 15 (102–210) ^{3*#}	170 ± 10 (126–234) [*]	100 ± 4 (74–116) [*]	410 ± 13 (374–392)	471 ± 15 (432–529) ^{2*#}	39 ± 18 (–42–88)
Swim 200 m freestyle (swim duration range 2 min 52 s — 5 min 3 s), $n = 10$							
92.4 ± 5.3 (59–105) [*]	670 ± 45 (569–1010) [*]	113 ± 3 (76–142)	160 ± 17 (140–182)	102 ± 6 (78–142) ^{**}	382 ± 11 (344–432)	470 ± 8 (429–501) [*]	45 ± 10 (15–95)
Swim, 10 min freestyle (350–550 m), $n = 10$							
95.9 ± 6.4 (85–114) ^{2*}	645 ± 46 (528–703) ^{2*}	115 ± 6 (80–152) ^{2*}	129 ± 10 (86–162) ^{2*2*2*}	109 ± 7 (83–137) ^{2*}	358 ± 17 (186–392) ^{3*♦}	532 ± 19 (403–797) ^{3*3*2*}	71 ± 9 (36–90) ^{2*}

Table compiled by the authors based on their own data

Note: * — $p < 0.05$; ^{2*} — $p < 0.01$; ^{3*} — $p < 0.001$ — significance of differences between baseline and post-swim values; ♦ — $p < 0.05$; ^{2♦} — $p < 0.01$; ^{3♦} — $p < 0.001$ — comparison after 10 min swims (350–450 m) freestyle and 25 m backstroke; ■ — $p < 0.05$; ^{2■} — $p < 0.01$ — comparison after 10 min swims (350–450 m) freestyle and 25 m front crawl; # — $p < 0.05$ — comparison after 25 m backstroke swims and 25 m front crawl swims; data are presented as the mean value and standard error of the mean $M \pm m$, in parentheses — ranges of minimum and maximum values.

⁵ World Health Organization (WHO). Body mass index (BMI). URL: <https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/body-mass-index> (access data 07.10.2025).

statistically significant decrease in the PQ interval was recorded. The duration of myocardial repolarization remained within normal limits in 8 out of 10 swimmers; however, its frequency-corrected QTc value significantly exceeded normal limits (Table 1).

Thus, the most significant changes in the conduction of the bioelectrical signal in the myocardium were observed in swimmers during prolonged 10-min swims ($p < 0.01$). After short-distance swims, a slowing of conduction from the sinus node to the atrioventricular node was noted (an increase in PQ relative to the baseline, $p < 0.001$) (Table 1). Conversely, during prolonged swims conduction accelerated (a decrease in PQ relative to baseline, $p < 0.01$) (Table 1). The corrected QTc interval increased in proportion to the duration of the swims. The significance of the increase in this parameter after 10-min swims compared to 25 m swims was $p < 0.01$ relative to the baseline, $p < 0.001$.

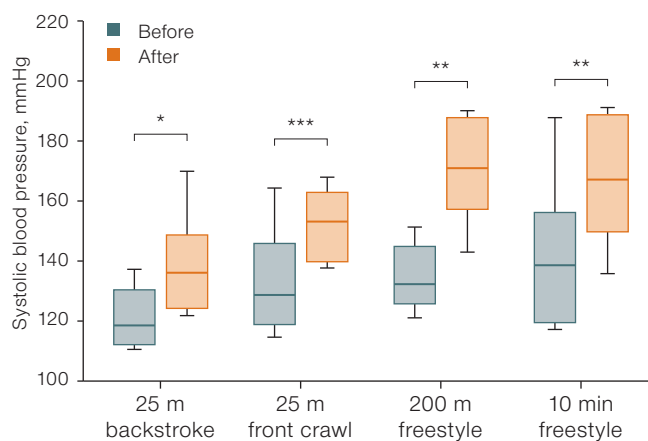


Figure prepared by the authors based on their own data

Fig. 1. Systolic blood pressure levels before and after swims at various distances

Note: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$ — statistical significance of differences between baseline and post-swim values.

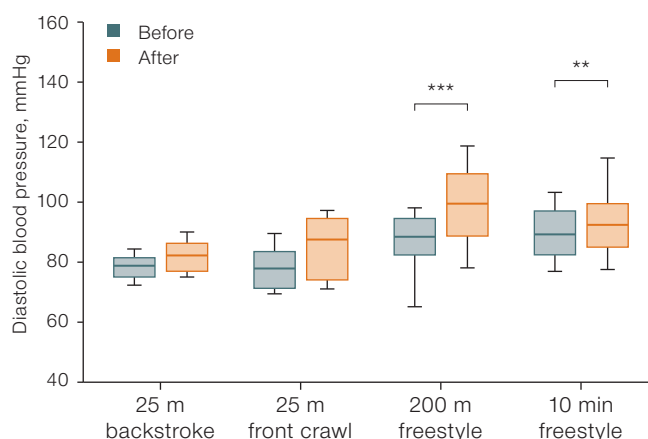


Figure prepared by the authors based on their own data

Fig. 2. Diastolic blood pressure levels (mmHg) before and after swims at various distances

Note: ** — $p < 0.01$, *** — $p < 0.001$ — significance of differences between baseline and post-swim values.

Changes in blood pressure levels under the influence of swims of varying duration in open cold water

At baseline rest, systolic blood pressure levels exceeded the optimal level (140 mmHg) in 3 individuals, and diastolic blood pressure (above 90 mmHg) was higher in 2 individuals. After the 25 m freestyle swim, the mean systolic pressure levels increased significantly (pre-swim 131.2 ± 2.6 mmHg, post-swim — 143.3 ± 4.1 mmHg ($p < 0.05$)) (Fig. 1). They remained within normal limits only in 18% of athletes. After the 25 m backstroke swim, systolic and diastolic pressure levels also increased; however, their values varied within normal limits in 50% of participants. The systolic pressure level after the 25 m front crawl swim significantly increased among the entire group (pre-swim — 131.2 ± 2.6 mmHg; post-swim — 147.9 ± 2.5 mmHg; $p < 0.001$). No statistically significant change in diastolic pressure after short-distance swims (25 m) was detected (Fig. 2).

After long swims of 200 m and 400–450 m, the mean blood pressure parameters increased significantly more than after short distances. At the same time, in 3 out of 10 swimmers, systolic and diastolic pressure levels remained within normal limits. In 2 athletes, systolic levels and in one athlete, diastolic levels significantly exceeded normal limits (systolic parameters exceeded 190 mmHg, and diastolic — 102 mmHg, respectively) (Fig. 1).

Changes in capillary blood glucose levels under the influence of swims of varying duration in open cold water

In all examined athletes, glucose levels before food intake did not exceed normal values (4.3–6.1 mmol/L). In some athletes, glucose levels before swims were slightly above normal. After 25 m freestyle swims, glucose levels increased in 85% of athletes (group mean pre-swim

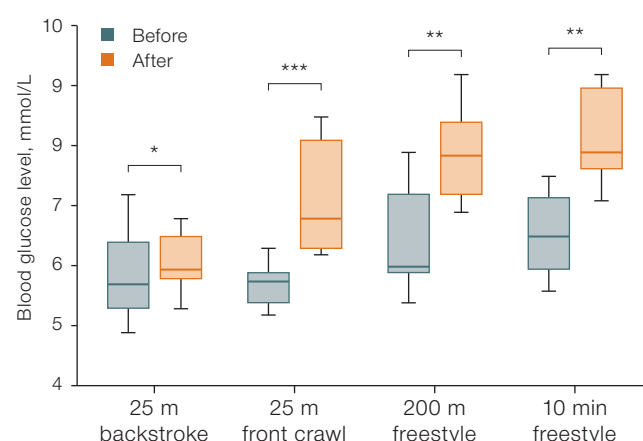


Figure prepared by the authors based on their own data

Fig. 3. Capillary blood glucose levels before and after swims at various distances

Note: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$ — significance of differences between baseline and post-swim values.

5.7 ± 0.1 , post-swim — 6.9 ± 0.1 ; $p < 0.05$); during 25 m backstroke swims, increased glucose levels were noted in 70% of subjects (group mean pre-swim 5.9 ± 0.2 , post-swim — 6.9 ± 0.2 ; $p < 0.05$). After swims of 200 m and 350–450 m (10-min swim), glucose levels increased in 9 out of 10 examined individuals. The mean glucose level before the swim was 6.2 ± 0.2 , post-swim — 8.2 ± 0.25 ; $p < 0.01$ (Fig. 3).

Changes in sensorimotor reaction time under the influence of swims of varying duration in open cold water

After short-distance swims (25 m) backstroke and free-style, the simple sensorimotor reaction time to a light stimulus changed insignificantly based on the group mean. According to individual indicators, it increased slightly in some swimmers and, conversely, decreased in others (3 athletes) (Table 2).

After the 200 m swim, this indicator increased statistically significantly in 6 out of 11 examined individuals, while it changed insignificantly at rest. After the 10-min swim, an increase in reaction time was observed statistically significantly in 9 out of 10 subjects (mean pre-swim — 263 ± 10.1 ms, post-swim — 328 ± 21.3 ms; $p < 0.01$) (Table 2).

DISCUSSION

The analysis of anthropometric indicators in amateur athletes adapted to cold-water swimming showed that the group mean did not exceed normal values. At the same time, in swimmers adapted to long-distance swims (10-min swim of 350–400 m), the body mass index was either at the upper limit of normal or even higher. The noted shifts are presented in the works of other authors [3].

The BMI indicator is an indirect criterion for the presence of adipose tissue in the body; in addition, it is internationally recognized as a marker of obesity and overweight⁶ [4, 5]. At the same time, in athletically built individuals, BMI may be associated with developed muscle tissue rather than with adipose tissue. Traditionally, in cold-water swimmers, an elevated body fat level is linked to protection against core hypothermia [6–9]. Furthermore, cooling of a larger body with greater mass

occurs more slowly [10]. However, contemporary studies have shown that the BMI of cold-water swimmers does not differ statistically significantly from that of classical swimmers in pools with normal water temperature [11].

Our data are consistent with the established concepts. Swimmers participating in short-distance swims under the extreme conditions of ice-cold water may have a low BMI, even near the lower limit of normal. However, only athletes with a sufficiently high BMI can withstand long-distance swims.

Our analysis of fasting-morning blood-glucose levels did not reveal individuals with hyperglycemia among cold-water swimmers. However, based on group means, capillary blood glucose concentration increased statistically significantly after the swims. During short-distance swims, the indicator was elevated in 80% of swimmers. After long-distance swims, an increase in glucose was observed in 90% of the examined individuals. A significant decrease in glucose levels in swimmers after the swims was not noted.

The increase in blood glucose levels during swims under competitive conditions in extremely cold water may be stress-induced [13–15]. Stress is considered an adaptive response of the body to a strong disturbing environmental stimulus [15]. The increased activity of the sympathoadrenal system in response to a set of stress factors not only increases the volume of substrate for oxidation in the form of glucose but also stimulates the activity of non-shivering thermogenesis. The latter is a mechanism of accelerated heat production through increased metabolic activity in brown (or beige in humans) adipose tissue. This occurs through the activation of β_2 -adrenergic receptors (β_2 AR) in brown adipocytes by the sympathoadrenal system, increasing cAMP levels with subsequent expression of the mitochondrial membrane protein thermogenin (uncoupling protein 1, UCP1). UCP1 allows energy to be dissipated as heat instead of producing ATP [16]. As previous studies have shown, adaptation to swimming in low-temperature water (from 0°C to +2°C) leads to a decrease in blood levels of adrenocorticotrophic hormone (ACTH) and cortisol, while catecholamines remain at high levels [17]. Currently, there exists evidence that, in addition to fatty acid oxidation during thermogenesis activation in brown adipocytes, glucose utilization also increases [18–20].

Table 2. Simple sensorimotor reaction time, ms

Swim 25 m backstroke, <i>n</i> = 13		Swim 25 m freestyle, <i>n</i> = 24		Swim 200 m freestyle, <i>n</i> = 11		Swim 10 min, 350–400 m, freestyle, <i>n</i> = 10	
Before swim	After swim	Before swim	After swim	Before swim	After swim	Before swim	After swim
257 ± 6.4	250 ± 7.3	251 ± 6.8	247 ± 6.2	245 ± 6.4	256 ± 10.3	263 ± 10.1	$328 \pm 21.3^{**}$

Table compiled by the authors based on their own data

Note: ** — $p < 0.01$ — significance of differences between baseline and post-swim values; data are presented as the mean value and standard error of the mean $M \pm m$.

⁶ Thompson WR, Gordon NF, Pescatello LS. The American college of sports medicine's guidelines for exercise testing and prescription. Baltimore, MD: Williams & Wilkins; 2010. URL: <https://acsm.org/education-resources/books/guidelines-exercise-testing-prescription/> (access data 07.10.2025).

However, as recent studies convincingly demonstrate [21], the primary oxidation substrates for both shivering and non-shivering thermogenesis are lipids (their share in heat production is 50%), 30% comes from muscle glycogen, 10% is provided by proteins, and only 10% by plasma glucose. This may explain the persistence of elevated blood glucose levels after swims of 200 m and 400–450 m (10-min swim).

The simple sensorimotor reaction time (SSRT) was used to assess the combined influence of competitive stress, the cold factor, and physical load on the functional state of the neuromuscular system. According to the results obtained, during 25 m swims (swim duration 15–30 s), no statistically significant changes were detected overall for the group. The stress impact (competitive and cold stress) is regarded as the main factor affecting the nervous system during short-distance swims. This is confirmed by the increased levels of capillary blood glucose. Moderate activation of the stress-implementing sympathoadrenal system can either accelerate or slow down processes in the CNS, depending on the body's stress resilience [22].

After the 200 m swim (swim durations from 2 min 51 s to 5 min 2 s), our analysis of individual indicators demonstrated a statistically significant slowing of SSRT in half of the examined individuals, while no significant changes were detected in the other half. During this swim, body cooling is likely to affect the speed of nervous processes, neuromuscular transmission, muscle response, and the overall sensorimotor reaction duration (especially in swimmers with swim times exceeding 3 min).

A more pronounced, statistically significant slowing of SSRT based on the group mean was identified during the 10-min swim. A slowing of SSRT was detected in 9 out of 10 swimmers. The primary probable factor determining this slowing is body cooling. For instance, some studies have shown that prolonged exposure to low temperatures impairs memory and reduces attention, affecting reaction speed and decision making. Sufficiently long immersion in cold water leads to a drop in brain temperature by fractions of a degree, causing cognitive impairments due to slowed neural conduction and synaptic transmission mechanisms [23–25].

Our analysis of the dynamics of cardiovascular system indicators revealed a dependence of their changes on the duration of exposure to cold water. The baseline blood pressure values, on average for the group, corresponded to age norms. During 25 m swims, systolic pressure increased statistically significantly for the group as a whole, while diastolic pressure increased only slightly. However, it should be noted that in two examined individuals, it exceeded normal limits: systolic pressure increased above 160 mmHg, and diastolic pressure reached 104 mmHg. During short swims, the main factor increasing blood pressure is likely to be the activation of the stress-implementing sympathoadrenal system. During longer swims of 200 m and 400–450 m, in addition to the stress response, protective reactions against heat loss contribute to increased blood

pressure, specifically sustained prolonged constriction of peripheral vessels, which increases peripheral resistance. A decrease in systolic pressure with a simultaneous increase in diastolic pressure, a reduction in pulse pressure, combined with a decrease in heart rate, was observed in one swimmer. Such a bodily response to the considered load should be considered unfavorable.

ECG analysis at baseline revealed slowed conduction through the atria in 66% of the examined swimmers. In clinical practice, this finding is considered unfavorable, being associated with signs of atrial chamber enlargement and atrial conduction block. It is also viewed as a risk factor in various clinical events, such as atrial fibrillation and ischemic stroke [26–28]. However, widening of the P wave may be due to a physiological condition often observed in athletes training in the aerobic power zone. In this case, P wave widening may be related to atrial hypertrophy resulting from adaptation to this type of activity [29, 30].

Cold-water swimming is an aerobic activity [31, 32] that places a significant load on the body's gas transport systems; therefore, it is quite reasonable to assume that it exerts a substantial load on the heart, contributing to myocardial hypertrophy and dilation of the heart chambers, including the atria.

Comparison of changes in myocardial conduction after 25 m backstroke and freestyle swims with facial immersion showed a more pronounced slowing of conduction during swims with facial immersion. This may be related to the activation of the so-called diving reflex and enhanced vagal parasympathetic influences on the sinus node of the heart [2]. It was also noted that the slowing of conduction, especially in the atria (P wave duration), immediately after swims varied among the examined individuals. In one swimmer, the conduction duration decreased; in three, it changed insignificantly, remaining within normal limits; in five, it exceeded the upper limit of normal (112–122 ms); and in eight, it significantly exceeded the norm (125–210 ms). We believe this is due to different myocardial reactivity to the influences of the *n. vagus* during the implementation of the diving reflex while swimming with facial immersion [33].

During longer swims of 200 m and 400–450 m, conduction velocity in the atria returns to the baseline levels. In our opinion, this is due to the onset of manifestation of sympathetic, along with parasympathetic, effects on the myocardium associated with cold stress. It is known that parasympathetic cholinergic effects are realized faster (<1 s from the onset of exposure) than sympathetic effects (>5 s from the onset of exposure) [34].

The increase in blood glucose levels during swims, especially during long-distance swims, indicates that the body is under stress. The duration of bioelectrical signal conduction through the ventricular myocardium progressively increases (increase in QTc) in accordance with the duration of exposure to water. This finding can be explained by several factors. First, electrolyte imbalances (e.g., hypokalemia) associated with physical exertion occur against the background of high catecholamine levels

[35] that are particularly elevated during cold stress in long swims. Second, the use of QT-prolonging medications or those stimulating metabolic changes. However, amateur athletes who participated in the swims had been previously warned about the undesirability of taking such substances. Third, cases of hereditary predisposition to QT interval prolongation, such as a mutation in the *KCNQ1* gene, should not be excluded. Physical exertion and high catecholamine levels associated with cold stress in such athletes may be a triggering factor [36]. It should also be noted that an increase in the corrected QT interval (QTc) was observed in all swims with facial immersion in water, but not in backstroke swims. In our opinion, this is further evidence that the diving reflex, activated during facial immersion while swimming in cold water, plays a significant role in generating risks for cardiac arrhythmias.

Our findings are supported by data obtained in a joint study by Slovenian and French researchers, who also observed an increase in the QTc interval during long-distance swims, although in a less extreme cold water (at 15°C). However, these researchers did not establish a direct correlation between the degree of body cooling and the increase in the QTc indicator. In our studies, a pronounced increase in QTc duration, exceeding 500 ms, was observed during the longest swims. A dependence between the conduction of the electrical signal in the myocardium and the degree of body temperature reduction may exist. Further research is needed to confirm this assumption. It can be hypothesized that the prolongation of the QTc interval arises from the cumulative effect of several factors, such as cold and competitive stress, electrolyte imbalance due to intense physical exertion, body cooling, cholinergic reactions of the diving reflex, and genetically determined characteristics of the organism [37, 38].

Slowing of myocardial conduction may also be associated with high myocardial reactivity to the cholinergic effects of the *n. vagus* [33] during the implementation of the diving reflex upon immersion in water. In this case, the additional factor of low temperature, causing body cooling, may act as a trigger for cardiac arrhythmias or even lead to cardiac arrest. Such cases have occurred in the practice of cold-water swimming and are described in the literature. Therefore, we believe that for permission to engage in cold-water swimming, an additional assessment of the response of the cardiovascular system to cold-water immersion should be conducted.

Hence, a dependence of hemodynamic and neurophysiological reactions on the duration of the swim has been demonstrated. At all distances, systolic blood pressure and glucose levels increased, while a pronounced slowing of the simple sensorimotor reaction and the most significant prolongation of the QTc interval were recorded during 10-min swims. This complements the theory of a stepped stress response to cold and physical exertion and integrates cardiac, metabolic, and regulatory changes of the neuromuscular system into a unified functional model.

Our results indicate the role of the diving reflex: swims with facial immersion in ice-cold water cause a more pronounced slowing of conduction and prolongation of the QTc interval than backstroke swims. This clarifies the mechanism of “autonomic conflict” (simultaneous activation of sympathetic and parasympathetic systems) as a risk factor in arrhythmias during cold-water immersion. Similar mechanisms have been also discussed in the international literature on autonomic conflict and arrhythmias during cold water immersion.

Our results may be of interest to cardiologists and functional diagnostic physicians. The data obtained demonstrate the necessity of introducing an additional criterion into the medical clearance for sports cold-water swimming, i.e., the response of the cardiovascular system to cold-water immersion. They can be used in categorizing competition distances and regulating their sequence in event programs; developing protocols for admission, monitoring, and rehabilitation of winter swimmers; and creating educational programs for coaches and beginners on safely entering cold-water swimming.

The limitations of our study are related to the small sample size (24 individuals) and the predominance of well-adapted amateur athletes, thus impeding direct extrapolation of the results to completely non-adapted beginners. Future research should compare swims in cold water with results from swims in a comfortable-temperature water. This would allow for a clearer separation of the contributions of chronic aerobic training and the cold factor itself to the formation of baseline ECG changes (widening of P and QTc). It should also be noted that we assessed the effects of hypothermia on myocardial conduction and nervous system processes indirectly, based on QTc and SSRT time, which requires confirmation in studies with direct monitoring of core body temperature. It would also be important to evaluate the effect of body composition (fat mass, subcutaneous adipose tissue thickness). In addition, the role of the fat component in heat conservation and tolerance of long distances was assessed only via BMI, thus requiring further research. Studying polymorphisms in genes encoding potassium channel proteins associated with QT prolongation appears promising. This could identify individuals with a potential risk of QTc prolongation under conditions of cold-water swimming.

CONCLUSIONS

1. Slowing of electrical impulse conduction through the atria (P wave duration > 0.11 ms, exceeding the upper limit of normal) was established at baseline rest in 67% of the examined swimmers; after short-distance swims (25 m), P wave duration significantly exceeded the upper normal limit in 85% of the examined.

2. A direct relationship was identified between the QTc indicator and both the distance and the duration of its completion. During the 10-min swim, an increase in QTc interval duration was observed in all swimmers. In 3 out of 10 athletes, QTc was at the upper limit of normal, while

in 5 athletes, it significantly exceeded the upper normal limit (QTc > 500 ms).

3. Comparison of myocardial conduction after short-distance swims (25 m) front crawl with facial immersion vs. backstroke swims revealed a statistically significantly more pronounced slowing of biopotential conduction through the atria and ventricles of the myocardium. This is likely due to the activation of the diving reflex and enhanced cholinergic influences of the *n. vagus* on the myocardium.

4. A significant increase in systolic blood pressure was established after swims at all distances; a

statistically significant increase in diastolic blood pressure based on the group mean was observed only after the 10-min swim.

5. A statistically significant increase in blood glucose levels based on the group mean after swims at all distances compared to the baseline reflected the presence of stress even in swimmers adapted to cold-water swimming.

6. An increase in the simple sensorimotor reaction time to a light stimulus relative to the baseline was identified after prolonged 10-min swims of 350–450 m, reflecting a slowing of processes in the central and peripheral nervous systems.

References

1. Podyacheva E, Zemlyanukhina T, Shadrin L, Baranova T. Features of hemodynamics of pulmonary circulation during the diving reflex. *Biological Communications*. 2020;65(3):244–51. <https://doi.org/10.21638/spbu03.2020.304>
2. Nordine M, Schwarz A, Bruckstein R, Gunga HC, Opatz O. The Human Dive Reflex During Consecutive Apnoeas in Dry and Immersive Environments: Magnitude and Synchronicity. *Frontiers in Physiology*. 2022;12:725361. <https://doi.org/10.3389/fphys.2021.725361>
3. Tipton M, Montgomery H. The experience of drowning. *Medico-Legal Journal*. 2022;90(1):17–26. <https://doi.org/10.1177/00258172211053127>
4. World Health Organization (WHO) Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *The Lancet*. 2004;363(9403):157–63. [https://doi.org/10.1016/S0140-6736\(03\)15268-3](https://doi.org/10.1016/S0140-6736(03)15268-3)
5. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. *JAMA*. 2012;307(5):491–7. <https://doi.org/10.1001/jama.2012.39>
6. Kenny J, Cullen S, Warrington GD. The “Ice-Mile”: Case Study of 2 Swimmers’ Selected Physiological Responses and Performance. *International Journal of Sports Physiology and Performance*. 2017;12(5):711–4. <https://doi.org/10.1123/ijspp.2016-0323>
7. Notley SR, Mitchell D, Taylor NAS. A century of exercise physiology: concepts that ignited the study of human thermoregulation. Part 4: evolution, thermal adaptation and unsupported theories of thermoregulation. *European Journal of Applied Physiology*. 2024;124(1):147–218. <https://doi.org/10.1007/s00421-023-05262-9>
8. Stephens JM, Halson SL, Miller J, Slater GJ, Chapman DW, Askew CD. Effect of Body Composition on Physiological Responses to Cold-Water Immersion and the Recovery of Exercise Performance. *International Journal of Sports Physiology and Performance*. 2018;13(3):382–9. <https://doi.org/10.1123/ijspp.2017-0083>
9. Tikuisis P. Prediction of survival time at sea based on observed body cooling rates. *Aviation, Space and Environmental Medicine*. 1997;68(5):441–8.
10. Tarlochan F, Ramesh S. Heat transfer model for predicting survival time in cold water immersion. *Biomedical Engineering: Applications, Basis and Communications*. 2005;17(4):159–66. <https://doi.org/10.4015/S1016237205000251>
11. Knechtle B, Barkai R, Hill L, Nikolaidis PT, Rosemann T, Sousa CV. Influence of Anthropometric Characteristics on Ice Swimming Performance-The IISA Ice Mile and Ice Km. *International Journal of Environmental Research and Public Health*. 2021;18(13):6766. <https://doi.org/10.3390/ijerph18136766>
12. Sancini A, Ricci S, Tomei F, Pacchiarotti A, Nardone N, Ricci P, et al. Work related stress and blood glucose levels. *Annali di Igiene*. 2017;29(2):123–33. <https://doi.org/10.7416/ai.2017.2139>
13. Song G, Liu X, Lu Z, Guan J, Chen Y, Li Y, et al. Relationship between stress hyperglycaemic ratio (SHR) and critical illness: a systematic review. *Cardiovascular Diabetology*. 2025;24(1):188. <https://doi.org/10.1186/s12933-025-02751-3>
14. Bosiaci M, Tarnowski M, Misiakiewicz-Has K, Lubkowska A. The Effect of Cold-Water Swimming on Energy Metabolism, Dynamics, and Mitochondrial Biogenesis in the Muscles of Aging Rats. *International Journal of Molecular Sciences*. 2024;25(7):4055. <https://doi.org/10.3390/ijms25074055>
15. Knechtle B, Stjepanovic M, Knechtle C, Rosemann T, Sousa CV, Nikolaidis PT. Physiological Responses to Swimming Repetitive “Ice Miles”. *Journal of Strength and Conditioning Research*. 2021;35(2):487–94. <https://doi.org/10.1519/JSC.0000000000002690>
16. Paulo E, Wu D, Wang Y, Zhang Y, Wu Y, Swaney DL, et al. Sympathetic inputs regulate adaptive thermogenesis in brown adipose tissue through cAMP-Salt inducible kinase axis. *Scientific Reports*. 2018;8(1):11001. <https://doi.org/10.1038/s41598-018-29333-6>
17. Leppäluoto J, Westerlund T, Huttunen P, Oksa J, Smolander J, Dugue B, et al. Effects of long-term whole-body cold exposures on plasma concentrations of ACTH, β -endorphin, cortisol, catecholamines and cytokines in healthy females. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2008;68:145–53. <https://doi.org/10.1080/00365510701516350>
18. Raiko JRH, Saari T, Orava J, Savisto N, Parkkola R, Haarpanta-Solin M, et al. Changes in electrocardiogram parameters during acute nonshivering cold exposure and associations with brown adipose tissue activity, plasma catecholamine levels, and brachial blood pressure in healthy adults. *Physiological Reports*. 2021;9(3):e14718. <https://doi.org/10.14814/phy2.14718>
19. Wakabayashi H, Sakaue H, Nishimura T. Recent updates on cold adaptation in population and laboratory studies, including cross-adaptation with nonthermal factors. *Journal of Physiological Anthropology*. 2025;44(1):7. <https://doi.org/10.1186/s40101-025-00387-6>
20. Wang Z, Ning T, Song A, Rutter J, Wang QA, Jiang L. Chronic cold exposure enhances glucose oxidation in brown adipose tissue. *The EMBO Reports*. 2020;21(11):e50085. <https://doi.org/10.15252/embr.202050085>
21. Chen W, Xu Z, You W, Zhou Y, Wang L, Huang L, et al. Cold exposure alters lipid metabolism of skeletal muscle through HIF-1 α -induced mitophagy. *BMC Biology*. 2023;21:27. <https://doi.org/10.1186/s12915-023-01514-4>

22. Scotto CR, Petitcollin F, Dupuy O, Casiez G, Dugué B, Toussaint L. Arm cooling selectively impacts sensorimotor control. *Journal of Neurophysiology*. 2024;131(4):607–18. <https://doi.org/10.1152/jn.00196.2023>
23. Jones DM, Bailey SP, Roelands B, Buono MJ, Meeusen R. Cold acclimation and cognitive performance: a review. *Autonomic Neuroscience*. 2017;208:36–42. <https://doi.org/10.1016/j.autneu.2017.11.004>
24. Taylor L, Watkins SL, Marshall H, Dascombe BJ, Foster J. The impact of different environmental conditions on cognitive function: a focused review. *Frontiers in Physiology*. 2016;6:372. <https://doi.org/10.3389/fphys.2015.00372>
25. Khan AM, Finlay JM, Clarke P, Sol K, Melendez R, Judd S, et al. Association between temperature exposure and cognition: a cross-sectional analysis of 20,687 aging adults in the United States. *BMC Public Health*. 2021;21(1):1484. <https://doi.org/10.1186/s12889-021-11533-x>
26. Massin SZ, Denham N, Kakarla J, Hans AK, Vigmond E, Chauhan VS. Left atrial deceleration outperforms regional conduction velocity in predicting arrhythmia recurrence following atrial fibrillation ablation. *Heart Rhythm O2*. 2025;6(7):928–39. <https://doi.org/10.1016/j.hroo.2025.03.020>
27. Istolahti T, Eranti A, Huhtala H, Lyytikäinen LP, Kähönen M, Lehtimäki T, et al. The prevalence and prognostic significance of interatrial block in the general population. *Annals of Medicine*. 2020;52(3–4):63–73. <https://doi.org/10.1080/07853890.2020.1731759>
28. Chen LY, Ribeiro ALP, Platonov PG, Cygankiewicz I, Soliman EZ, Gorenek B, et al. P Wave Parameters and Indices: A Critical Appraisal of Clinical Utility, Challenges, and Future Research—A Consensus Document Endorsed by the International Society of Electrocardiology and the International Society for Holter and Noninvasive Electrocardiology. *Circulation. Arrhythmia and Electrophysiology*. 2022;15(4):e010435. <https://doi.org/10.1161/CIRCEP.121.010435>
29. Konieczny K, Banks L, Osman W, Glibbery M, Connelly KA, Yan AT, et al. Prolonged P wave duration is associated with right atrial dimensions, but not atrial arrhythmias, in middle-aged endurance athletes. *Journal of Electrocardiology*. 2019;56:115–20. <https://doi.org/10.1016/j.jelectrocard.2019.07.002>
30. Banks L, Al-Mousawy S, Altaha MA, Konecny KM, Osman W, Currie KD, et al. Cardiac remodeling in middle-aged endurance athletes: relation between signal-averaged electrocardiogram and LV mass. *American Journal of Physiology. Heart and Circulatory Physiology*. 2021;320(1):H316–22. <https://doi.org/10.1152/ajpheart.00602.2020>
31. Manolis AS, Manolis SA, Manolis AA, Manolis TA, Apostolaki N, Melita H. Winter Swimming: Body Hardening and Cardiorespiratory Protection Via Sustainable Acclimation. *Current Sports Medicine Reports*. 2019;18(11):401–15. <https://doi.org/10.1249/JSR.0000000000000653>
32. Bosiacki M, Tarnowski M, Misiakiewicz-Has K, Lubkowska A. The Effect of Cold-Water Swimming on Energy Metabolism, Dynamics, and Mitochondrial Biogenesis in the Muscles of Aging Rats. *International Journal of Molecular Sciences*. 2024;25(7):4055. <https://doi.org/10.3390/ijms25074055>
33. Baranova TI, Rybyakova TV, Dmitrieva MO, Anisimov DA, Tarasova MS, Ogannisyan MG. Specifics of reaction of human cardiovascular system to immersion in cold water. *Extreme Medicine*. 2023;25(4):106–15 (In Russ.). <https://doi.org/10.47183/mes.2023.053>
34. Nunan D, Sandercock GR, Brodie DA. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *PACE*. 2010;33(11):1407–17. <https://doi.org/10.1111/j.1540-8159.2010.02841.x>
35. Jha S. A Case Report on QTc Prolongation: Understanding the Medication Risks and Electrolyte Imbalance. *Cureus*. 2022;14(1):e21421. <https://doi.org/10.7759/cureus.21421>
36. Lazzarini E, Lodrini AM, Arici M, Bolis S, Vagni S, Panella S, et al. Stress-induced premature senescence is associated with a prolonged QT interval and recapitulates features of cardiac aging. *Theranostics*. 2022;12(11):5237–57. <https://doi.org/10.7150/thno.70884>
37. Faivre-Rampant V, Hingrand C, Mezanger A, Saloux E, Ollitrault P, Alvado S, et al. Cardiac electrical and functional activity following an outdoor cold-water swimming event. *Journal of Thermal Biology*. 2024;125:103996. <https://doi.org/10.1016/j.jtherbio.2024.103996>
38. Wu X, Larsson HP. Insights into Cardiac IKs (KCNQ1/KCNE1) Channels Regulation. *International Journal of Molecular Sciences*. 2020;21(24):9440. <https://doi.org/10.3390/ijms21249440>

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PARAMETERS OF BONE TISSUE METABOLISM AND HORMONAL CHARACTERISTICS IN ADOLESCENT ATHLETES WITH FUNCTIONAL HYPOGONADISM

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Introduction. The syndrome of relative energy deficiency (RED-S) in male athletes can lead to the development of functional hypogonadism (FH), characterized by testosterone deficiency and impairments in bone tissue remodeling processes.

Objective. Assessment of the hormonal profile and parameters of bone tissue metabolism in blood serum in adolescent athletes in the setting of FH presence/absence.

Materials and methods. A single-center, cross-sectional study involved 50 adolescent athletes aged 15–18 years (median age 16.5 [15.9; 16.9] years), all members of Russian national teams in three kinds of sport (Greco-Roman wrestling, judo, and football). The participants were divided into two groups based on FH presence (according to the total testosterone level ≤ 12 nmol/L). The FH group included 12 athletes; the comparison group without FH comprised 38 athletes. Serum levels of parathyroid hormone, 25-hydroxyvitamin D, and bone metabolism markers (osteocalcin, C-terminal telopeptide of type I collagen (β -CrossLaps), N-terminal propeptide of type I procollagen), as well as total alkaline phosphatase activity, were determined in all athletes. To assess the hormonal profile, serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), inhibin B, total testosterone, and leptin were investigated. Body composition assessment was performed using bioelectrical impedance analysis. Sexual development was assessed according to the Tanner rating. Statistical data processing was carried out using the Statistica v. 10.0 software package (StatSoft Inc.; USA).

Results. Athletes with FH exhibited a statistically significantly higher percentage of body fat compared to their peers with normal total testosterone levels (13.5 [8.1; 19.9]% and 10.1 [9.0; 12.2]%, respectively, $p = 0.034$). Conversely, the groups did not differ statistically significantly in terms of lean body mass (58.3 [46.5; 82.0] kg and 61.9 [55.4; 67.0] kg, respectively, $p = 0.742$). Athletes with androgen deficiency showed a lower level of LH compared to athletes without FH (1.6 [0.9; 2.9] IU/L and 2.7 [1.7; 3.4] IU/L, respectively, $p = 0.031$), while FSH and inhibin B levels were comparable (3.4 [2.2; 4.2] IU/L and 3.5 [2.1; 5.7] IU/L, respectively, $p = 0.547$; 176.5 [147.0; 248.6] pg/mL and 194.5 [166.0; 231.6] pg/mL, respectively, $p = 0.586$). The levels of bone metabolism markers in athletes with FH did not differ statistically significantly from those in the group of athletes with normal testosterone levels.

Conclusions. The FH development in adolescent male high-performance athletes can be accompanied by gonadostat dysfunction and preserved Sertoli cell function. Androgen deficiency in athletes is associated with an increase in body fat percentage, without changes in lean body mass. In young male athletes, FH does not have a negative impact on bone tissue remodeling processes, given the absence of statistically significant changes in bone metabolism parameters.

Keywords: adolescent athletes; sports medicine; functional hypogonadism; testosterone; osteocalcin; β -CrossLaps; vitamin D

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ПАРАМЕТРЫ МЕТАБОЛИЗМА КОСТНОЙ ТКАНИ И ГОРМОНАЛЬНЫЕ ХАРАКТЕРИСТИКИ НЕСОВЕРШЕННОЛЕТНИХ СПОРТСМЕНОВ С ФУНКЦИОНАЛЬНЫМ ГИПОГОНАДИЗМОМ

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Введение. Синдром относительного дефицита энергии у спортсменов мужского пола может приводить к развитию функционального гипогонадизма (ФГ), сопровождающегося дефицитом тестостерона и нарушениями процессов ремоделирования костной ткани.

Цель. Оценка гормонального профиля и параметров метаболизма костной ткани в сыворотке крови у несовершеннолетних спортсменов в зависимости от наличия ФГ.

Материалы и методы. Проведено одноцентровое одномоментное исследование, включившее 50 юных спортсменов 15–18 лет (средний возраст 16,5 [15,9; 16,9] года), входящих в состав сборных команд Российской Федерации по трем видам спорта (греко-римская борьба, дзюдо, футбол) и разделенных на две группы в зависимости от наличия ФГ (по уровню общего тестостерона ≤ 12 нмоль/л). В группу с ФГ включены 12 спортсменов; в группу сравнения без ФГ — 38 атлетов. У спортсменов в сыворотке крови определяли уровни паратиреоидного гормона, 25-гидроксивитамина D, маркеров метаболизма костной ткани (остеокальцин, С-концевой телопептид коллагена 1-го типа (β -CrossLaps), N-терминальный пропептид проколлагена 1-го типа), а также активность общей щелочной фосфатазы. Для оценки гормонального профиля исследовали уровни лютеинизирующего гормона (ЛГ), фолликулостимулирующего гормона (ФСГ), ингибина В, общего тестостерона и лептина в сыворотке крови. Оценка композиционного состава тела проведена методом биоимпедансного анализа. Половое развитие оценивали по классификации Tanner. Статистическую обработку данных проводили с использованием пакета прикладных программ Statistica v. 10.0 (StatSoftInc., США).

Результаты. У спортсменов с ФГ выявлено статистически значимо большее процентное содержание жировой ткани в организме по сравнению со сверстниками с нормальным уровнем общего тестостерона (13,5 [8,1; 19,9] и 10,1 [9,0; 12,2]% соответственно, $p = 0,034$, в то время как по количеству тощей массы тела исследуемые группы статистически значимо не различались (58,3 [46,5; 82,0] и 61,9 [55,4; 67,0] кг соответственно, $p = 0,742$). У спортсменов с андрогенным дефицитом выявлен более низкий уровень ЛГ по сравнению со спортсменами без ФГ (1,6 [0,9; 2,9] и 2,7 [1,7; 3,4] МЕ/л соответственно, $p = 0,031$) при сопоставимых значениях ФСГ и ингибина В (3,4 [2,2; 4,2] и 3,5 [2,1; 5,7] МЕ/л соответственно, $p = 0,547$; 176,5 [147,0; 248,6] и 194,5 [166,0; 231,6] пг/мл соответственно, $p = 0,586$). Уровни маркеров метаболизма костной ткани у спортсменов с ФГ статистически значимо не отличались от группы спортсменов с нормальным уровнем тестостерона.

Выводы. Развитие ФГ у несовершеннолетних высококвалифицированных спортсменов мужского пола сопровождается нарушением функции гонадостата и сохраненной функцией клеток Сертоли. Андрогенный дефицит у спортсменов ассоциирован с увеличением содержания жировой ткани в организме, но не сопровождается изменением количества тощей массы тела. У юных спортсменов мужского пола ФГ не оказывает негативного влияния на процессы ремоделирования костной ткани, учитывая отсутствие статистически значимых изменений параметров метаболизма костной ткани.

Ключевые слова: юные спортсмены; спортивная медицина; функциональный гипогонадизм; тестостерон; остеокальцин; β -CrossLaps; 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

High-quality and balanced nutrition in terms of quantity and composition is essential for full recovery after training, adaptation to intense physical loads, and prevention of sports injuries. Insufficient dietary intake of energy substrates required to maintain the body's optimal function during sports can lead to the development of the condition referred to as the Relative Energy Deficiency in Sport (RED-s) syndrome [1]. The risk of energy deficiency in male athletes is highest in endurance (marathon, cycling) and weight-category sports (judo, boxing, wrestling, and other combat sports) due to extremely high energy expenditure (endurance sports) and stringent body weight requirements (combat sports) in the aforementioned athletic specializations [2].

In adolescent males, RED-s can be accompanied by androgen deficiency as part of the development of functional hypogonadism (FH) [1, 3]. Furthermore, a decrease in testosterone levels in young male athletes can be observed in cases of overtraining syndrome [4]. It was previously shown that the development of functional hypothalamic amenorrhea in female athletes negatively affects bone structure and mineral density (BMD), being a leading risk factor in low-energy fractures [1, 5]. At the same time, the influence of FH on microarchitecture and BMD in male athletes is less pronounced [1, 3]. However, a number of studies demonstrate the negative impact of androgen deficiency in adult athletes on BMD, reduced performance, as well as increased fatigue and sexual dysfunction [6, 7].

In clinical practice, bone functional status is assessed using bone metabolism markers [8]. The high risk of low-energy fractures in athletes underscores the relevance of studying bone metabolism markers and their relationship with hormonal parameters in adolescent male athletes with FH, given the lack of data on this topic in the literature.

This study is aimed at assessing the hormonal profile and parameters of bone tissue metabolism in the blood serum of adolescent athletes depending on the presence or absence of FH.

MATERIALS AND METHODS

A single-center, cross-sectional study involved 50 male adolescent athletes of three sports specializations: Greco-Roman wrestling, *n* = 14; judo, *n* = 22; football, *n* = 14. The age of participants ranged 15–18 years (median age 16.5 [15.9; 16.9] years); all reached sexual maturity (Tanner IV–V). Due to the completed sexual maturity of the examined athletes, diagnostic criteria for hypogonadism in adult males were applied.

All athletes were divided into two groups based on the presence of functional hypogonadism (FH), diagnosed by a decrease in total testosterone level ≤ 12 nmol/L [9]. The group with FH included 12 athletes (median age 16.2 [15.9; 16.9] years); the comparison group with normal total testosterone levels comprised 38 athletes (median age 16.6 [15.9; 16.9] years).

Anthropometric measurements of the adolescent athletes included: height and body weight measurement, calculation of body mass index (BMI). BMI was assessed for the specific age and sex, 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). Assessment of the stage of sexual maturity of adolescent athletes was conducted according to the Tanner scale [10].

The inclusion criteria for participation in the study were male athletes from Russian national teams, aged 15–18 years, with achieved sexual maturity (Tanner IV–V). The inclusion criterion for the study group was the presence of FH (decreased total testosterone level ≤ 12 nmol/L). The inclusion criterion for the comparison group was the absence of FH (normal total testosterone level).

Blood was collected in the morning from a peripheral vein after overnight fasting. Serum levels of osteocalcin (Roche, Switzerland), C-terminal telopeptide of type I collagen (β-CrossLaps; Roche, Switzerland), 25-hydroxyvitamin D (25(OH)D; Roche, Switzerland), N-terminal propeptide of type I procollagen (P1NP; Roche, Switzerland), and parathyroid hormone (PTH; Roche, Switzerland) (ng/mL) were determined in all adolescent athletes using an electrochemiluminescence immunoassay. The osteocalcin/β-CrossLaps ratio was also calculated [11]. Leptin (ng/mL), luteinizing hormone (LH, IU/L), follicle-stimulating hormone (FSH, IU/L), and inhibin B (pg/mL) levels were measured in serum using an enzyme-linked immunosorbent assay (ELISA) (BenderMedSystems, Austria). The serum level of total testosterone (nmol/L) was determined by immunoassay on a Lazurit automated analyzer (USA). Total alkaline phosphatase (ALP) activity in serum (U/L) was measured using a kinetic colorimetric method.

Statistical data processing was performed using the Statistica v. 10.0 software package (StatSoft Inc.; USA). Since the studied quantitative parameters had a non-normal distribution (according to the Kolmogorov–Smirnov test), all data are presented as the median (*M*_e)

Table. Clinical characteristics of the studied athlete groups

Investigated parameter, units of measurement	FH Group (<i>n</i> = 12)	Group without FH (<i>n</i> = 38)	Statistical significance level, <i>p</i>
Age, years	16.2 [15.9;16.9]	16.6 [15.9;16.9]	0.682
Height, m	1.70 [1.63; 1.85]	1.73 [1.67; 1.80]	0.733
Height SDS	-0.41 [-1.11; 1.58]	0.32 [-0.85; 0.94]	0.785
Body weight, kg	74.6 [55.2; 105.1]	70.2 [62.3; 75.6]	0.716
BMI, kg/m ²	24.6 [20.1; 29.9]	23.1 [21.4; 24.1]	0.785
BMI SDS	1.04 [-1.65; 2.50]	0.91 [-0.53; 1.35]	0.665
Sport:			
judo	5 (46%)	17 (44%)	0.851
Greco-Roman wrestling	6 (50%)	8 (22%)	0.051
football	1 (4%)	13 (34%)	0.081

Table was compiled by the authors based on their own data

Note: *n* — number of athletes; FH — functional hypogonadism; SDS — standard deviation score; BMI — body mass index; BMI SDS — standard deviation score of body mass index.

with the upper and lower quartiles [Q_1 ; Q_3], corresponding to the 25th and 75th percentiles of the distribution. The Mann–Whitney U test was used to assess the statistical significance of differences in quantitative characteristics. Qualitative characteristics are presented as proportions (%) with absolute values indicated. To assess differences between qualitative characteristics, contingency tables were constructed followed by evaluation using Pearson's chi-square test (χ^2) with Yates' correction. Correlation analysis was conducted using Spearman's rank correlation coefficient. The statistical level of significance for differences was accepted at $p \leq 0.05$.

RESULTS

The studied groups did not differ statistically significantly and were comparable in age ($p = 0.682$) and basic anthropometric parameters (Table). The distribution by sport type in the groups with FH and without androgen deficiency is presented in the table.

An analysis of gonadotropin levels in adolescent male athletes did not reveal statistically significant differences between the studied groups in FSH levels (3.4 [2.2; 4.2] IU/L and 3.5 [2.1; 5.7] IU/L for the groups with FH and without androgen deficiency, respectively, $p = 0.547$). However, adolescents with androgen deficiency showed statistically significantly lower levels of LH and total testosterone compared to athletes without FH (1.6 [0.9; 2.9] IU/L and 2.7 [1.7; 3.4] IU/L, respectively, $p = 0.031$, and 9.3 [8.4; 11.0] nmol/L versus 21.6 [15.6; 25.9] nmol/L, respectively, $p < 0.001$).

When assessing the functional activity of Sertoli cells in the testicular tissue, no statistically significant differences were found between the studied groups of adolescent male athletes in inhibin B levels (176.5 [147.0; 248.6] pg/mL and 194.5 [166.0; 231.6] pg/mL, respectively).

Adolescent athletes with FH were characterized by statistically significantly higher leptin levels of 2.1 [0.8; 5.1] ng/mL compared to the group of athletes without testosterone deficiency — 0.8 [0.5; 1.5] ng/mL ($p = 0.017$).

Correlation analysis revealed a statistically significant inverse relationship between leptin levels and total testosterone ($r_s = -0.48$; $p < 0.05$), as well as between inhibin B and FSH ($r_s = -0.51$; $p < 0.001$).

Assessment of bone tissue metabolism parameters established that the presence of androgen deficiency did not lead to changes in the values of bone formation markers (P1NP, osteocalcin, ALP) or bone resorption markers (β -CrossLaps) compared to peers without FH.

Thus, in the group with FH, the osteocalcin level was 71.0 [38.1; 104.5] ng/mL, P1NP 245.1 [193.4; 516.9] ng/mL, total ALP 150.7 [108.9; 196.7] U/L, β -CrossLaps 1.51 [1.28; 2.3] ng/mL. In athletes

without androgen deficiency, the osteocalcin level was 68.0 [53.7; 88.0] ng/mL, P1NP 322.7 [260.7; 496.0] ng/mL, total ALP 147.2 [114.9; 181.5] U/L, β -CrossLaps 1.82 [1.41; 2.29] ng/mL.

The osteocalcin/ β -CrossLaps ratio in athletes with FH was 38.3 [35.8; 42.2] and did not differ statistically significantly from the values in athletes without androgen deficiency (42.3 [32.5; 49.4]; $p = 0.375$). The obtained values of bone metabolism parameters corresponded to reference intervals developed for athletes [11].

Assessment of vitamin D status in all athletes, regardless of FH presence, revealed low 25(OH)D levels, corresponding to a deficiency state in the body [12]. The studied groups did not differ statistically significantly in PTH levels (4.8 [3.3; 7.0] pmol/L and 4.3 [3.6; 5.4] pmol/L, respectively, $p = 0.699$).

Correlation analysis showed negative relationships between lean body mass in the athletes and levels of P1NP ($r_s = -0.56$; $p < 0.001$), osteocalcin ($r_s = -0.42$; $p = 0.002$), and β -CrossLaps ($r_s = -0.43$; $p = 0.002$).

Assessment of body composition in adolescent athletes with FH (Fig.) revealed a statistically significantly higher percentage (13.5 [8.1; 19.9]% vs 10.1 [9.0; 12.2]%, $p = 0.034$) and absolute (11.7 [5.9; 16.4] kg vs 6.6 [5.9; 8.4] kg, $p = 0.021$) content of body fat compared to the group without androgen deficiency. The groups did not differ statistically significantly in the amount of lean and skeletal muscle mass (Fig.).

Correlation analysis revealed a positive relationship between LH and leptin with the percentage of body fat in athletes ($r_s = 0.58$; $p < 0.001$ and $r_s = 0.38$; $p = 0.006$, respectively), and a negative relationship between the level of total testosterone and the absolute amount of body fat in young athletes ($r_s = -0.34$; $p < 0.05$).

DISCUSSION

The development of RED-s is associated with endocrine disorders and changes in bone metabolism, which increase the risk of fractures in both male and female athletes [11]. Currently, decreased levels of total or free testosterone are considered by the International Olympic Committee expert panel as a significant risk factor for the development of RED-s syndrome and impaired bone remodeling in adolescent male athletes [1].

Our study demonstrated that in adolescent male athletes with FH, the levels of key bone metabolism markers did not differ statistically significantly from peers without testosterone deficiency. This indirectly supports clinical data on the limited influence of androgen deficiency on the mechanisms of impaired bone remodeling in male athletes. The values of the studied bone metabolism markers in both investigated groups ranged within the reference intervals proposed for adolescent athletes with achieved or near-achieved sexual maturity [13]. The osteocalcin/ β -CrossLaps ratio may

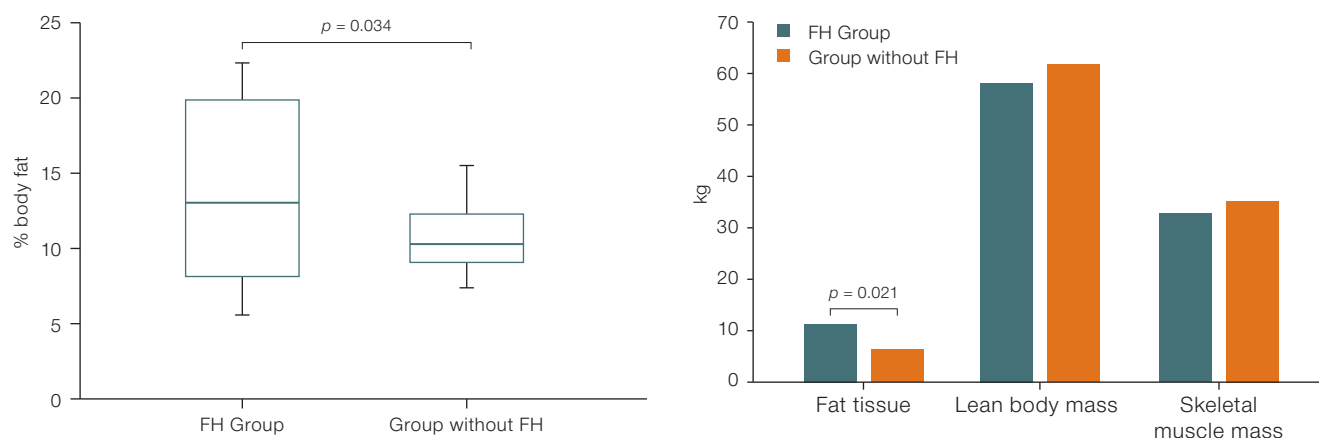


Figure prepared by the authors based on original data

Fig. Parameters of body composition in adolescent athletes depending on the presence of functional hypogonadism

reflect the balance between bone synthesis and re-sorption in athletes [11]. In our study, the ratio of bone marker indices did not differ statistically significantly between the groups with FH and without androgen deficiency and corresponded to the reference intervals proposed by Nikitina et al. [11]. The data obtained may indirectly indicate an absence of bone tissue remodeling disorders in adolescent athletes, regardless of the presence of hypogonadism syndrome.

According to Areta et al., the frequency of low-energy fractures in male athletes is three times lower compared to females [14]. Disruption of bone tissue remodeling processes in females is caused by hypoestrogenism in the setting of functional hypothalamic amenorrhea, whereas the development of FH in males leads to a slight decrease in circulating estrogen levels due to reduced aromatase activity [3]. Given the physiologically lower baseline level of estrogens in men compared to women, the development of pronounced disorders in bone tissue remodeling processes is not typical for the former group.

Assessment of gonadostat function in athletes with androgen deficiency revealed a statistically significantly lower level of LH compared to the group without FH. Reduced pulsatile secretion of LH by adenohypophyseal gonadotrophs is described as one of the key mechanisms in the development of functional hypogonadism within the chronic energy deficiency syndrome in athletes [1]. Normally, by interacting with Leydig cells, LH stimulates testosterone synthesis, and a decrease in LH level causes the androgen deficiency identified in the study group with FH. Prolonged and uncompensated hypogonadism syndrome in males may lead to a decrease in the number of Leydig cells, a reduction in LH receptors on their surface with decreased sensitivity, thus contributing to reduced fertility [15].

Inhibin B is secreted by Sertoli cells under the influence of FSH, acting as a regulator of its secretion via negative feedback. Thus, inhibin B can be used as a marker of spermatogenesis and Sertoli cell function; thus, its decrease is observed in male patients with various spermatogenesis disorders (oligospermia, azoospermia, etc.). Inhibin B levels reach peak values during puberty, around 12–17 years of age [16].

In our study, the levels of inhibin B and FSH in the investigated groups of young athletes did not differ statistically significantly, indicating no influence of androgen deficiency on Sertoli cell function in adolescent athletes. The study design did not allow us to assess the persistence and duration of the identified androgen deficiency in the athletes. Therefore, the absence of changes in Sertoli cell functional activity may be due to a short-term or transient decrease in testosterone levels.

According to Meyer et al., a low body fat percentage (<5%) in adolescent male athletes based on body composition analysis can be considered an indirect sign of insufficient energy intake [17]. None of the athletes we examined had a low body fat percentage. In young athletes with androgen deficiency, a statistically significantly greater amount of body fat was found compared to their peers without FH. The obtained results are consistent with data from other researchers, demonstrating a reduction in body fat in males with normal or elevated testosterone levels [18].

The amount of body fat in adolescent male athletes positively correlated with the level of circulating leptin. This adipokine (adipose tissue hormone) is currently considered an endogenous regulator and modulator of reproductive system functions; however, in male athletes, leptin apparently reflects the dynamics of body fat content to a greater extent and does not have a significant influence on gonadostat function [19]. In our study, the

median leptin level in adolescent male athletes without androgen deficiency was lower compared to the general population norm, which is associated with a lower body fat content in athletes.

At the same time, the studied groups did not differ significantly in the amount of skeletal muscle mass, which is not consistent with the data of Aksenova et al., demonstrating a direct relationship between testosterone levels and an increase in skeletal muscle mass in adolescent athletes [20].

Our study has one important limitation, since the vast majority of athletes in both study groups had vitamin D deficiency or insufficiency, which could have influenced the levels of the analyzed bone metabolism markers. It is known that prolonged, uncompensated vitamin D deficiency leads to increased PTH secretion to maintain calcium homeostasis, which occurs through an intensification of resorptive processes in bone tissue and is accompanied by an increase in bone resorption markers in serum [21]. Assessing bone metabolism markers against a background of adequate vitamin D sufficiency in male athletes with FH would allow for a more precise determination of the influence of androgen deficiency on bone remodeling processes.

Within the scope of this work, we did not investigate the influence of sports type, level of athletic skill, or stage of the training cycle on the studied bone metabolism markers due to the restricted sample size of athletes with FH. Furthermore, the serum level of total testosterone was determined only once as part of the comprehensive medical examination, which does not allow for conclusions regarding the persistence and duration of the identified androgen deficiency.

It can be hypothesized that the decrease in total testosterone levels in adolescent athletes is an adaptive mechanism to high physical loads or short-term energy deficiency (during rapid weight loss in combat sports athletes), which explains the absence of associations between androgen deficiency and body composition parameters or bone metabolism markers.

Given that a decrease in testosterone levels can serve as a marker of overtraining syndrome in athletes [4], special attention should be paid to young athletes with FH to clarify their tolerance to training loads in this group.

Further investigation of the characteristics of the hormonal profile and bone remodeling markers and their influence on BMD may have important practical significance for developing an individualized approach to diagnosing bone remodeling disorders and stratifying the risks of low-energy fractures in adolescent male athletes with functional hypogonadism.

CONCLUSION

Functional hypogonadism in adolescent male athletes does not lead to changes in bone tissue metabolism parameters and does not exert a negative influence on bone remodeling processes. The development of androgen deficiency in young athletes is associated with impaired gonadostat function, rather than with changes in the functional activity of Sertoli cells. The androgen deficiency identified in male athletes with FH is associated with an increase in body fat content, although not being accompanied by a reduction in lean and skeletal muscle mass. Considering the functional nature of androgen deficiency and its lack of impact on bone remodeling processes and fertility prognosis, in our opinion, there is no need to impose restrictions on sports participation for young athletes with FH. However, to ensure the continuation of productive training, special attention must be paid to preventing the development of overtraining syndrome in these young athletes. Furthermore, in this athlete group, it is advisable to continue monitoring hormonal indicators over time, assessing additional markers of overtraining.

Clarifying the persistence of the identified gonadostat dysfunction and androgen deficiency in male athletes with FH requires further research to determine the strategy for their medical and biological support.

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. Burke LM, Close GL, Lundy B, Mooses M, Morton JP, Tenforde AS. Relative energy deficiency in sport in male athletes: a commentary on its presentation among selected groups of male athletes. *International Journal of Sport Nutrition and Exercise Metabolism*. 2018;28(4):364–74. <https://doi.org/10.1123/ijsnem.2018-0182>
3. 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>
4. Kostina OV, Galova EA, Presnyakova MV. Features of the effect of hormones on increasing the risk of sports injuries and the development of overtraining syndrome in children

- and adolescent athletes (review of literature). *Russian Clinical Laboratory Diagnostics*. 2025;70(2):89–96 (In Russ.).
<https://doi.org/10.51620/0869-2084-2025-70-2-89-96>
5. 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>
 6. Hackney AC, Lane AR, Register-Mihalik J, O'Leary CB. Endurance exercise training and male sexual libido. *Medicine and Science in Sports and Exercise*. 2017;49(7):1383–8.
<https://doi.org/10.1249/MSS.0000000000001235>
 7. Hooper DR, Tenforde AS, Hackney AC. Treating exercise-associated low testosterone and its related symptoms. *The Physician and Sportsmedicine*. 2018;46(4):427–34.
<https://doi.org/10.1080/00913847.2018.1507234>
 8. Kiseleva NG, Taranushenko TE, Golubenko NK. Diagnosis of osteoporosis at an early age. *Medical Council*. 2020;1:179–86 (In Russ.).
<https://doi.org/10.21518/2079-701X-2020-1-186-193>
 9. Dedov II, Mokrysheva NG, Melnichenko GA, Rozhivanov RV, Kamalov AA, Mkrtumyan AM, et al. Draft of Russian Clinical Practice Guidelines «Male hypogonadism». *Obesity and metabolism*. 2021;18(4):496–507 (In Russ.).
<https://doi.org/10.14341/omet12817>
 10. 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.
 11. Nikitina KI, Safonov LV, Abramova TF, Nikitina TM, Abduvosidov KhA. Features of Bone Metabolism in Cycling Athletes. *Modern Problems of Science and Education*. 2022;6(1) (In Russ.).
<https://doi.org/10.17513/spno.32268>
 12. Pigarova EA, Rozhinskaya LYa, Belaya JE, Dzeranova LK, Karonova TL, Ilyin AV, et al. Russian Association of Endocrinologists recommendations for diagnosis, treatment and prevention of vitamin D deficiency in adults. *Problems of Endocrinology*. 2016;62(4):60–84 (In Russ.).
<https://doi.org/10.14341/probl201662460-84>
 13. Isaeva EP, Okorokov PL, Stolyarova SA, Kluchnikov SO, Zybkin IV, Isaev MR, et al. Bone metabolism markers in young high-performance athletes. *Extreme Medicine*. 2025;27(3):384–91 (In Russ.).
<https://doi.org/10.47183/mes.2025-256>
 14. Areta JL, Taylor HL, Koehler K. Low energy availability: history, definition and evidence of its endocrine, metabolic and physiological effects in prospective studies in females and males. *European Journal of Applied Physiology*. 2021;121(1):1–21.
<https://doi.org/10.1007/s00421-020-04516-0>
 15. Pichugova SV. Comparative characteristics of endocrine profile in males with infertility as well as in adolescents with varicocele. *Obstetrics, Gynecology and Reproduction*. 2021;15(2):156–65 (In Russ.).
<https://doi.org/10.17749/2313-7347/ob.gyn.rep.2021.155>
 16. Crofton PM, Evans AE, Groome NP, Taylor MR, Holland CV, Kelnar CJ. Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clinical Endocrinology*. 2002;56(2):215–21.
<https://doi.org/10.1046/j.0300-0664.2001.01448.x>
 17. Meyer NL, Sundgot-Borgen J, Lohman TG, Ackland TR, Stewart AD, Maughan RJ, et al. Body composition for health and performance: a survey of body composition assessment practice carried out by the Ad Hoc Research Working Group on Body Composition, Health and Performance under the auspices of the IOC Medical Commission. *British Journal of Sports Medicine*. 2013;47(16):1044–53.
<https://doi.org/10.1136/bjsports-2013-092561>
 18. Berkovskaya MA, Elmurzaeva AA, Edaev ALA, Selakhov TYu, Tokaev HM, Gurova ID. The impact of physical exercise on the endocrine system in men: A review. *Consilium Medicum*. 2024;26(4):263–68 (In Russ.).
<https://doi.org/10.26442/20751753.2024.4.202697>
 19. 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>
 20. Aksenova NV, Mangushev TA, Okorokov PL, Babaeva EV, Zybkin IV. Effect of testosterone in young ice-hockey players on hematological, biochemical parameters and the level of physical performance. *Sports Medicine: Research and Practice*. 2023;13(1):80–7 (In Russ.).
<https://doi.org/10.47529/2223-2524.2023.1.8>
 21. Mokrysheva NG, Eremkina AK, Mirnaya SS, Kovaleva EV. Challenges in differential diagnosis between primary and secondary forms of hyperparathyroidism. *Obesity and Metabolism*. 2017;14(3):48–53 (In Russ.).
<https://doi.org/10.14341/omet2017348-53>

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ANALYSIS OF BLOOD PARAMETERS IN ATHLETES TAKING INTO ACCOUNT HEMATOCRIT LEVELS

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Introduction. Assessment of hematological parameters in professional athletes requires a special approach. This includes analysis of hematocrit levels, which can be quite high due to their significant oscillations during intense muscular activity and under conditions of relative rest. **Objective.** Study of the additional influence of various hematocrit levels on morphological and biochemical blood parameters, determined within the framework of the comprehensive medical examination (CME) of athletes.

Materials and methods. A retrospective study was conducted using data from athletes' medical records. A total of 26,413 hematological parameters obtained during the CME of athletes from Russian national teams were analyzed. Hematocrit was calculated using an automatic Mindray BC-6200 analyzer as the ratio of red blood cell volume to total blood volume. Nonparametric reference intervals for hematocrit, hemoglobin, red blood cell count, and mean corpuscular volume were constructed according to the CLSI EP28-A3c standard. Associations between hematocrit and blood parameters (lipid and protein profiles, glucose, oxygen transport system, sodium, potassium, magnesium, and platelets) were assessed using Spearman's rank correlations within extreme sub-samples ($\leq p25$ and $\geq p75$ of hematocrit). Only correlations with Spearman's coefficient above 0.5 were considered significant. Comparisons of parameter distributions between percentile groups of hematocrit were performed pairwise using the nonparametric Wilcoxon test with an FDR correction.

Results. Irrespective of sex, within the normal hematocrit range (25–75 percentiles), a strong positive correlation is observed with hemoglobin levels ($r_s = 0.836$) and red blood cell count ($r_s = 0.631$). For hematocrit values above the 75th percentile, a strong positive correlation is maintained with hemoglobin concentration ($r_s = 0.801$) and red blood cell count ($r_s = 0.603$). Regarding hematocrit values below the 25th percentile, a near-significant correlation was identified with red blood cell count ($r_s = 0.437$), as well as a strong positive correlation with hemoglobin ($r_s = 0.839$).

Conclusions. Across different percentile ranges of hematocrit, significant differences in numerous blood biochemical parameters were identified. Thus, the higher the hematocrit level, the higher the concentrations of the recorded parameters in the blood serum. This underscores the importance of taking into account, inter alia, the possible influence of the hematocrit level when evaluating hematological parameters in athletes.

Keywords: professional athletes; morphological and biochemical blood composition; percentile gradations; hematocrit; comprehensive medical examination; high-performance sport

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АНАЛИЗ ПОКАЗАТЕЛЕЙ КРОВИ У СПОРТСМЕНОВ С УЧЕТОМ УРОВНЯ ГЕМАТОКРИТА

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Введение. Оценка гематологических параметров у профессиональных спортсменов требует особого подхода, в том числе к анализу уровня гематокрита, который может быть достаточно высоким, с учетом его осцилляции при напряженной мышечной деятельности и в условиях относительного покоя.

Цель. Изучение дополнительного влияния различных уровней гематокрита на показатели морфологического и биохимического состава крови, определяемые в рамках углубленного медицинского обследования (УМО) спортсменов.

Материалы и методы. Проведено ретроспективное исследование по данным медицинских карт спортсменов. Проанализировано 26 413 гематологических показателей, полученных при проведении УМО спортсменов сборных команд России. Гематокрит рассчитывался на автоматическом анализаторе Mindray BC-6200 как отношение объема эритроцитов к объему крови. Для гематокрита, гемоглобина, количества эритроцитов и среднего объема эритроцитов были построены непараметрические референсные интервалы по стандарту CLSI EP28-A3c. Ассоциации между гематокритом и показателями крови (липидный и белковый профиль, глюкоза, кислородтранспортная система, натрий, калий, магний, тромбоциты) оценивались ранговыми корреляциями Спирмена в крайних подвыборках ($\leq p25$ и $\geq p75$ гематокрита). Значимыми считались только корреляции при значениях коэффициента

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Спирмена выше 0,5. Сравнение распределений показателей между центильными группами гематокрита проводилось попарно непараметрическим критерием Вилкоксона с FDR-поправкой.

Результаты. Независимо от пола в нормальном диапазоне показателя гематокрита (25–75 перцентилей) наблюдается его тесная положительная взаимосвязь с показателями гемоглобина ($r_s = 0,836$) и количеством эритроцитов ($r_s = 0,631$). При значениях гематокрита выше 75 перцентилей тесную положительную взаимосвязь с ним проявляет концентрация гемоглобина ($r_s = 0,801$) и количество эритроцитов ($r_s = 0,603$). Что же касается значений показателя гематокрита ниже 25 перцентилей, то здесь была выявлена его близкая к достоверной взаимосвязь с количеством эритроцитов ($r_s = 0,437$), а также сильная положительная взаимосвязь с гемоглобином ($r_s = 0,839$).

Выводы. В разных перцентильных диапазонах гематокрита выявлены достоверные различия многих биохимических показателей крови: чем выше гематокрит, тем выше концентрации регистрируемых параметров в сыворотке крови. Это обуславливает необходимость при анализе и оценке гематологических показателей у спортсменов учитывать, помимо целого ряда других факторов, возможное дополнительное влияние величины гематокрита.

Ключевые слова: профессиональные спортсмены; морфологический и биохимический состав крови; перцентильные градации; гематокрит; углубленное медицинское обследование; спорт высших достижений

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: авторы заявляют, что одобрение комитетом по этике не требовалось, поскольку были проанализированы обезличенные данные медицинских карт (архивные материалы) и люди непосредственно не участвовали в исследовании.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The issues concerning the necessity of a special approach to the analysis and evaluation of hematological parameters in professional athletes, in particular with additional consideration of the hematocrit level, remain poorly studied [1–5]. The values of the vast majority of hematological parameters reflect their concentration in blood serum, the volume of which decreases during hypohydration. As a result, the concentration of substances contained therein increases accordingly [1, 6]. Hypohydration in athletes can arise from unreplenished fluid deficiency, hyponatremia, or hypoalbuminemia, which determine the osmotic and oncotic pressure of the blood [1].

According to Petibois et al. [7], when analyzing biochemical parameters in athletes, the phenomenon of plasma volume changes induced by physical exercise should be taken into account. Cooling, psychological stress, nutrition, hydration, as well as prolonged and intense exercise can significantly alter hemoconcentration during physical exertion, challenging comparison of data obtained by different studies. In order to address this problem in the future, the factor of hemoconcentration must be taken into account. Indeed, substantial biochemical shifts may be observed after correction for exercise-induced plasma volume changes.

Baranovskaya et al. demonstrated that in a homogeneous group of male athletes during a delayed post-exercise period, specifically 40 h after the last training session, distinct differences in the values of certain blood biochemical parameters were established, which were significantly associated with different levels of hematocrit [8]. In a study by Jacob et al., an attempt was made

to analyze the influence of the hematocrit parameter on biochemical and morphological blood parameters [9].

Currently, there exist at least four methods for determining hematocrit in laboratory settings, each with its own advantages and disadvantages [10]. This must be taken into account when conducting a comparative analysis of research results, especially when different methods for determining hematocrit are used (in venous or capillary blood, under conditions of relative rest or during intense muscular activity, etc.).

In this study, our aim was to investigate the influence of various hematocrit levels on the parameters of morphological and biochemical blood composition determined within the framework of the comprehensive medical examination (CME) of athletes.

To achieve this aim, the following tasks were set:

- to calculate percentile gradations of the hematocrit parameter in male and female athletes and to identify the relationship of its various percentile ranges with parameters of the morphological and biochemical blood composition;
- to determine the significance of differences in the analyzed set of parameters of morphological and biochemical blood composition across different percentile ranges of the hematocrit parameter.

MATERIALS AND METHODS

A retrospective study of medical record data of athletes was conducted. A total of 26,413 hematological results from the CME of athletes from Russian national teams were analyzed (49% female, 51% male; mean age — 20 ± 3 years). The following parameters were taken into account: hematological (hemoglobin, hematocrit, mean

corpuscular volume, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelet volume) and biochemical (levels of sodium, potassium, magnesium, iron in blood serum; concentrations of cholesterol, high-density and low-density lipoproteins, albumin, total protein, glucose, total cholesterol).

Descriptive characteristics were presented as median and interquartile range. Non-parametric reference intervals (2.5–97.5 percentile) for hematocrit, hemoglobin, red blood cell count, and mean corpuscular volume were constructed according to the CLSI EP28-A3c standard, with preliminary diagnostics and exclusion of outliers based on Cook’s distance (using the reference intervals package) [11, 12].

The threshold values of the 25th and 75th percentiles of hematocrit were used to stratify the sample into three groups: $\leq p25$, $p25-p75$, and $\geq p75$. All calculations were performed in R 4.x using dplyr, tidyr, broom, purrr, ggplot2, and flextable. The statistical significance level was two-sided ($\alpha = 0.05$). Multiple testing was controlled using the Benjamini–Hochberg method within each test family. Missing values were handled on a complete-case basis in each specific model/comparison; variables with zero variability were excluded.

Associations between hematocrit and blood parameters were assessed using Spearman’s rank correlations in the extreme subsamples ($\leq p25$ and $\geq p75$ of hematocrit) between hematocrit and each quantitative indicator (separately in each subsample). The calculation required

at least three observations and non-zero variance for both variables; in cases where these conditions were not met, the correlation for that pair was not calculated. Only correlation coefficients with a critical Spearman’s rank correlation coefficient (r_s) value of above 0.5 were considered significant [13].

Comparison of parameter distributions between hematocrit groups ($\leq p25$, $p25-p75$, $\geq p75$) was performed pairwise using the nonparametric Wilcoxon test with a FDR correction within the panel of biochemical/hematological markers [14]. Correlations for the central range ($p25-p75$) and the results of one-factor models on the full sample were used as a reference for comparison with estimates in the extreme quartiles and for calculating standardization coefficients.

RESULTS AND DISCUSSION

At the first stage, percentile gradations of red blood cell parameters were calculated. The data obtained are presented in Table 1. The interrelationships of hematocrit within its different percentile ranges with the other studied morphological and biochemical blood parameters were identified (Table 2).

Borderline health conditions in athletes begin to develop before the onset of clinical manifestations and before blood parameters begin to exceed the reference interval [6, 15, 16]. This explains the currency of the percentile approach to the analysis and assessment of blood parameters in professional athletes in sports

Table 1. Percentile gradations of hematocrit, hemoglobin, mean corpuscular volume, and red blood cell count in professional athletes

Parameter	n	Sex	Percentiles							
			p5	p10	p25	p50	p75	p85	p90	p95
Hematocrit, %	8757	F	35.74	38.65	39.47	40.25	41.15	43.01	43.86	44.0
	10,410	M	41.29	41.84	42.90	44.20	45.90	46.68	47.64	48.6
Hemoglobin, g/L	8757	F	115.00	118.80	125.0	130.8	136.40	139.3	141.5	144.30
	10,410	M	133.00	137.00	143.0	149.0	155.00	158.7	160.7	164.30
Red blood cell count, $\times 10^{12}/L$	8757	F	3.99	4.10	4.27	4.47	4.68	4.80	4.88	5.00
	10,410	M	4.54	4.66	4.85	5.06	5.28	5.40	5.49	5.62
Mean corpuscular volume, fL	8757	F	80.70	82.80	85.56	88.32	90.92	92.30	93.20	94.50
	10,410	M	81.29	83.07	85.40	87.78	90.03	91.39	92.20	93.50
Mean corpuscular HGB, pg	8757	F	26.01	27.00	28.20	29.30	30.30	30.80	31.15	31.65
	10,410	M	27.00	27.70	28.60	29.50	30.40	30.84	31.18	31.64
Mean corpuscular HGB concentration, g/dL	8757	F	31.50	31.80	32.50	33.19	33.73	34.03	34.24	34.51
	10,410	M	32.20	32.50	33.04	33.60	34.11	34.40	34.60	34.90

Table compiled by the authors based on their own data
Note: n — number of athletes; F — females; M — males; HGB — hemoglobin.

Table 2. Correlative relationships of the hematocrit parameter in different percentile ranges with the studied morphological and biochemical blood parameters

Parameter	Hematocrit values below the 25th percentile			Hematocrit values within 25–75 percentiles			Hematocrit values above the 75th percentile		
	<i>n</i>	<i>r_s</i>	<i>p</i>	<i>n</i>	<i>r_s</i>	<i>p</i>	<i>n</i>	<i>r_s</i>	<i>p</i>
Sodium, mmol/L	4653	0.044	0.003	9483	0.054	0.000	4735	-0.004	0.794
Hemoglobin, g/L	4788	0.839	0.000	9591	0.836	0.000	4788	0.801	0.000
HDL cholesterol, mmol/L	4651	0.046	0.002	9472	-0.154	0.000	4731	-0.069	0.000
Red blood cell count, $\times 10^{12}/L$	4788	0.437	0.000	9591	0.631	0.000	4788	0.603	0.000
Mean corpuscular volume, fL	4788	0.270	0.000	9591	-0.007	0.514	4788	0.076	0.000
Mean corpuscular HGB, pg	4798	0.179	0.000	9572	0.061	0.000	4797	0.041	0.005
Mean corpuscular HGB concentration, g/dL	4798	0.078	0.000	9572	0.137	0.000	4797	-0.040	0.005
Potassium, mmol/L	4656	0.089	0.000	9486	0.089	0.000	4737	-0.008	0.596
Magnesium, mmol/L	4652	0.080	0.000	9475	0.039	0.000	4729	0.004	0.769
Albumin, g/L	4648	0.076	0.000	9474	0.099	0.000	4731	0.001	0.962
Total protein, g/L	4665	0.104	0.000	9488	0.061	0.000	4740	0.048	0.001
Glucose, mmol/L	4658	0.026	0.077	9492	0.082	0.000	4743	-0.032	0.028
Total cholesterol, mmol/L	4652	0.112	0.000	9473	-0.093	0.000	4735	0.046	0.002
LDL cholesterol, mmol/L	4650	0.092	0.000	9471	0.000	0.985	4731	0.066	0.000
Iron, $\mu\text{mol}/L$	4727	0.216	0.000	9512	0.109	0.000	4747	0.084	0.000
Platelets, $\times 10^9/L$	4788	-0.095	0.000	9591	-0.132	0.000	4788	-0.004	0.800
Mean platelet volume, fL	4787	0.015	0.308	9589	0.016	0.107	4788	0.053	0.000

Table compiled by the authors based on their own data

Note: *n* — number of athletes; *r_s* — Spearman's correlation coefficient; HGB — hemoglobin; HDL — high-density lipoproteins.; LDL — low-density lipoproteins.; *p* — statistical significance level ($p \leq 0.05$).

medicine [6, 15, 16]. It is assumed that the optimal values of blood parameters in athletes, ensuring their optimal performance, fall within the 25–75 percentile range (Table 1) [15, 16].

During the research, statistically significant correlations were obtained between different percentile ranges of hematocrit and the blood parameters studied (Table 2). Independent of sex and under the normal hematocrit range (25–75 percentiles), a strong positive direct correlation was found with hemoglobin levels ($r_s = 0.836$; $p = 0.000$) and a moderate correlation with red blood cell count ($r_s = 0.631$; $p = 0.000$). For hematocrit values above the 75th percentile, a strong positive direct correlation was noted with hemoglobin concentration ($r_s = 0.801$; $p = 0.000$) and a moderate correlation with red blood cell count ($r_s = 0.603$; $p = 0.000$). Regarding hematocrit values below the 25th percentile, a statistically significant moderate positive correlation was found with red blood cell count ($r_s = 0.437$; $p = 0.000$),

as well as a strong positive correlation with hemoglobin ($r_s = 0.839$; $p = 0.000$).

The strong association of hematocrit values across all its percentile ranges with hemoglobin concentration and red blood cell count indicates that these parameters should always be recorded together. When distinct discrepancies arise between the percentile intervals of hematocrit, hemoglobin, and red blood cell count, blood tests should be rechecked. In this regard, the World Anti-Doping Agency includes both hemoglobin concentration and hematocrit level among other indirect parameters of erythropoiesis in doping control screening procedures, without attempting to separate these parameters [17].

Interpretation of red blood cell parameters, particularly hematocrit, in athletes requires consideration of dynamic shifts in plasma volume. Two opposing processes — hemoconcentration and hemodilution — significantly influence blood rheology and oxygen transport

Table 3. Pairwise comparisons of the studied blood parameters between groups of athletes with hematocrit values below the 25th percentile, within 25th–75th percentiles, and above the 75th percentile

Parameter	Percentiles						Statistical significance level		
	< 25		25–75		> 75				
	<i>n</i>	<i>M</i> [95% CI]	<i>n</i>	<i>M</i> (95% CI)	<i>n</i>	<i>M</i> (95% CI)	<i>p</i> ₁	<i>p</i> ₂	<i>p</i> ₃
Albumin, g/L	4648	43.09 [42.99; 43.19]	9474	43.96 [43.89; 44.04]	6719	44.69 [44.59; 44.79]	≤0.001	≤0.001	≤0.001
Total protein, g/L	4665	69.86 [69.74; 69.99]	9488	70.96 [70.88; 71.05]	6731	72.09 [71.98; 72.19]	≤0.001	≤0.001	≤0.001
Hemoglobin, g/L	4788	124.09 [123.88; 124.29]	9591	140.68 [140.55; 140.82]	4797	156.41 [156.24; 156.59]	≤0.001	≤0.001	≤0.001
Red blood cell count, ×10 ¹² /L	4788	4.33 [4.32; 4.33]	9591	4.79 [4.79; 4.80]	4797	5.29 [5.28; 5.30]	≤0.001	≤0.001	≤0.001
Iron, μmol/L	4727	14.61 [14.40; 14.82]	9512	17.59 [17.45; 17.73]	6846	19.49 [19.32; 19.67]	≤0.001	≤0.001	≤0.001
Mean corpuscular volume, fL	4788	86.94 [86.80; 87.08]	9591	88.05 [87.97; 88.12]	4797	88.27 [88.17; 88.37]	≤0.001	0.4	≤0.001
Mean corpuscular hemoglobin, pg	4798	28.77 [28.71; 28.83]	9572	29.40 [29.37; 29.43]	4797	29.60 [29.57; 29.64]	≤0.001	≤0.001	≤0.001
Mean corpuscular hemoglobin concentration, g/dL	4798	33.08 [33.05; 33.11]	9572	33.40 [33.38; 33.41]	4797	33.54 [33.52; 33.56]	≤0.001	≤0.001	≤0.001
Potassium, mmol/L	4656	4.08 [4.07; 4.09]	9486	4.16 [4.16; 4.17]	6730	4.17 [4.16; 4.17]	≤0.001	≤0.001	0.4
Magnesium, mmol/L	4652	0.83 [0.83; 0.83]	9475	0.84 [0.84; 0.84]	6716	0.84 [0.84; 0.85]	≤0.001	≤0.001	≤0.001
Sodium, mmol/L	4653	139.12 [139.06; 139.18]	9483	139.47 [139.43; 139.51]	6725	139.46 [139.41; 139.50]	≤0.001	≤0.001	0.8
Glucose, mmol/L	4658	4.89 [4.88; 4.91]	9492	4.98 [4.97; 4.99]	6753	4.94 [4.93; 4.96]	≤0.001	≤0.001	≤0.001
Mean platelet volume, fL	4787	8.88 [8.85; 8.91]	9589	8.89 [8.87; 8.91]	4797	8.98 [8.95; 9.01]	≤0.001	≤0.001	≤0.001
Platelets, ×10 ⁹ /L	4788	258.34 [256.86; 259.83]	9591	247.40 [246.37; 248.43]	4797	237.61 [236.29; 238.92]	≤0.001	≤0.001	≤0.001
HDL cholesterol, mmol/L	4651	1.74 [1.72; 1.75]	9472	1.60 [1.59; 1.61]	6718	1.10 [1.08; 1.11]	≤0.001	≤0.001	≤0.001
LDL cholesterol, mmol/L	4650	2.21 [2.19; 2.23]	9471	2.28 [2.27; 2.30]	6717	2.39 [2.37; 2.40]	≤0.001	≤0.001	≤0.001
Cholesterol, mmol/L	4652	4.37 [4.34; 4.39]	9473	4.34 [4.33; 4.36]	6730	4.31 [4.29; 4.33]	≤0.001	0.034	0.005

Table compiled by the authors based on their own data

Note: *n* — number of athletes; CI — confidence interval of the mean; *p* — significance of differences in blood parameters depending on hematocrit percentiles (*p*₁ — <25, >75; *p*₂ — <25, 25–75; *p*₃ — 25–75, >75).

function and must be considered key factors in evaluating data from laboratory monitoring.

Hemoconcentration is characterized by an increased concentration of cellular elements per unit volume of blood due to a decrease in plasma volume. This leads to an apparent or false elevation of hematocrit and hemoglobin concentrations. The main mechanisms underlying this phenomenon in athletes are dehydration due to sweating [18]; fluid shift into the interstitial space; “stress” hemoconcentration involving the release of catecholamines and cortisol in response to physical exertion that causes a transient reduction in plasma volume, possibly due to increased vascular tone and changes in permeability.

At the same time, hemodilution is the process of increasing blood plasma volume, leading to a decrease in the relative concentration of red blood cells and hemoglobin. This is the process of physiological adaptation, particularly pronounced in endurance-training athletes. The main mechanisms include adaptive hypervolemia (athletic hypervolemia), where the key roles are played by the activation of the renin–angiotensin–aldosterone system and increased vasopressin secretion in response to exercise [19]; expansion of plasma volume after a single bout of exercise.

Hemodilution improves the rheological properties of blood (reduces viscosity), facilitates cardiac output, and enhances cutaneous blood flow for thermoregulation. However, this underlies the phenomenon of “sports pseudoaemia”, when an adapted athlete with a normal total red blood cell mass and absolute hemoglobin content shows hematocrit and hemoglobin values per unit volume of blood at the lower limit of norm or slightly below. This condition is adaptive and does not require treatment [20]. It is critically important to differentiate this condition from true iron deficiency states.

The second stage of the work involved pairwise comparisons of the studied blood parameters among groups of athletes with hematocrit values below the 25th percentile, between the 25th–75th percentiles, and above the 75th percentile (Table 3). According to the obtained results, the parameters of protein, lipid, and carbohydrate metabolism differ statistically significantly across different percentile ranges of hematocrit. This illustrates the necessity of additionally considering the influence of hematocrit on biochemical blood parameters when carrying out their assessment in athletes.

Only the differences in mean corpuscular volume and mean platelet volume were found to be non-significant in the hematocrit range below the 25th percentile and between the 25th–75th percentiles. For hematocrit values between the 25th–75th percentiles and above the 75th percentile, the differences in blood sodium and potassium content parameters were non-significant.

During the differential analysis of the influence of hematocrit values on the biochemical composition of blood (Table 3), significant differences were established in the designated ranges of the hematocrit parameter,

as well as in hemoglobin and red blood cell concentrations. At the same time, an increase in serum iron levels in the setting of consistently high hematocrit has an important differential diagnostic significance. The most probable cause of such a combination is polycythemia vera, where clonal proliferation of erythroid cells is accompanied by ineffective erythropoiesis, altered hepcidin regulation, and intense iron turnover. This distinguishes polycythemia vera from secondary hypoxic erythrocytoses, which are characterized by normal or decreased iron levels due to its active utilization [21].

Furthermore, in cases of blood thickening due to dehydration, a simultaneous increase in the concentration of all plasma components, including iron, occurs. This is the state of relative hyperferremia, rather than a true increase in the total amount of iron in the body.

In addition, a small but statistically significant increase in total protein and albumin content at a hematocrit level above the 75th percentile is most likely associated with a decrease in the volume of the blood’s liquid part. However, it cannot be ruled out that this also confirms the influence of the body’s protein potential on hematopoiesis [1, 22]. A combined increase in hematocrit, total protein, and albumin under a normal or elevated sodium level is a classic sign of dehydration.

In our opinion, it is interesting that hemoconcentration is accompanied by worsening of lipid metabolism indicators (increased LDL levels and decreased HDL levels), while simultaneously showing a tendency to reduce the risk of thrombus formation due to decreased platelet content in the blood. During dehydration, plasma volume decreases, leading to a false (relative) increase in the concentration of all plasma components, including LDL. HDL levels may also falsely increase, although this effect is less pronounced. The combined alteration of the lipid profile (increase in low-density lipoproteins — LDL and decrease in high-density lipoproteins — HDL) and a reduction in platelet count (thrombocytopenia) in the setting of elevated hematocrit is a complex pathophysiological phenomenon characteristic of a number of conditions, primarily associated with chronic hypoxia [23, 24].

Thus, when analyzing and assessing the concentration of key biochemical blood composition indicators in athletes, it is necessary to additionally account for the hematocrit level and the possibility of associated shifts in the analyzed parameters, even within their normal ranges.

CONCLUSION

Interpretation of red blood cell parameters, particularly hematocrit, in athletes requires consideration of dynamic shifts in plasma volume. Two opposing processes — hemoconcentration and hemodilution — significantly affect blood rheology and oxygen transport function and should be considered key factors in evaluating laboratory monitoring data.

In professional athletes aged 17–23 years under relative rest conditions, the hematocrit level of venous blood, when measured using a Mindray BC-6200 analyzer, shows a strong positive correlation primarily with hemoglobin concentration and red blood cell count, regardless of sex. To avoid measurement errors, these indicators should be determined simultaneously. In cases where distinct discrepancies are

found in the percentile intervals of hematocrit, hemoglobin, and red blood cell count, they must be re-checked.

Statistically significant differences found between the values of individual blood parameters at different hematocrit levels justify the necessity of additionally considering this factor when analyzing and evaluating hematological indicators in athletes.

References

- Kulinenkov OS, Lapshin IA. *Biochemistry in Sports Practice*. Moscow: Sport Publishing House; 2018 (In Russ.). EDN: [YUODBG](#)
- Gavril'yeva KS, Handy MV, Solovyova MI, Vinokurova SP, Makharova NV. The influence of physical activity on the morphological composition of red blood in adolescents in Yakutia. *Doctor.Ru*. 2018;11(155):27–30 (In Russ.). <https://doi.org/10.31550/1727-2378-2018-155-11-27-30>
- Abdurakhmonov ZhS, Kuchkarova LS. The influence of physical activity on the blood profile in elite canoeists. *Scientific Review. Biological Sciences*. 2023;4:34–8 (In Russ.). <https://doi.org/10.17513/srbs.1339>
- Agishev AA, Fateev IS. Formation of sports results. Correlation of power and hemoglobin level. *Human Health, Theory and Methodology of Physical Education and Sports*. 2019;4(15):358–68 (In Russ.). EDN: [GNQPKG](#)
- Martykanova DS, Mavliev FA, Ibragimova MYa, Akhmetov II, Zhdanov RI. Hematological parameters of the blood of young men involved in cyclic and team sports. *Science and Sport: Modern Trends*. 2018;4(21):19–24 (In Russ.). EDN: [LSSZGD](#)
- Avdeeva MG, Aranson MV, Bezuglov EN, Bushueva TV, Vavaev AV, Vinogradov MA, et al. *Practical sports medicine for trainers*. Moscow: Sport Publ.; 2022 (In Russ.). EDN: [ZUICFF](#)
- Petibois C, Cazorla G, Poortmans JR, Déléris G. Biochemical aspects of overtraining in endurance sports: a review. *Sports Medicine*. 2002;32(13):867–78. <https://doi.org/10.2165/00007256-200232130-00005>
- Baranovskaya IB, Makarova GA, Bratova AV, Kholyavko YuA. Methodological approaches to the analysis of blood biochemical composition indicators in highly qualified athletes (using the de Ritis coefficient as an example). *Therapeutic Physical Education and Sports Medicine*. 2016;3(135):4–13 (In Russ.). EDN: [WAYGVB](#)
- Jacob M, Annaheim S, Boutellier U, Hinske C, Rehm M, Breymann C, et al. Haematocrit is invalid for estimating red cell volume: a prospective study in male volunteers. *Blood Transfusion*. 2012;10(4):471–9. <https://doi.org/10.2450/2012.0111-11>
- Akulov SA, Chistyakova IB, Fedotov AA. Methods for measuring blood hematocrit. *Volga Region Scientific Bulletin*. 2014;11-1(39):29–32 (In Russ.). EDN: [SZNZKX](#)
- Horowitz GL. *Defining, establishing, and verifying reference intervals in the clinical laboratory: Approved Guideline*. 3rd ed. Clinical and Laboratory Standards Institute; 2008.
- Hastie T, Tibshirani R, Friedman J. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. 2nd ed. New York: Springer; 2009.
- Hollander M, Wolfe DA, Chicken E. *Nonparametric Statistical Methods*. 3rd ed. Hoboken: John Wiley & Sons; 2014.
- Cardinali A, Nason GP. Costationarity of Locally Stationary Time Series Using costat. *Journal of Statistical Software*. 2013;55(1):1–36. <https://doi.org/10.18637/jss.v055.i01>
- Makarova GA, Kolesnikova NV, Skibitsky VV, Baranovskaya IB, Feshchenko VS, Bratova AV, et al. *Diagnostic potential of blood picture in athletes*. Moscow: Sport Publ.; 2020 (In Russ.). EDN: [TYZHNH](#)
- Grishina ZhV, Makarova GA, Chernukha SM, Feshchenko VS, Zholinsky AV. A special approach to the analysis and evaluation of red blood composition in athletes (using kayaking and canoeing as an example). *Sports Medicine: Science and Practice*. 2021;11(4):26–31 (In Russ.). <https://doi.org/10.47529/2223-2524.2021.4.10>
- Wozny M. The biological passport and doping in athletics. *Lancet*. 2010;376:79. [https://doi.org/10.1016/S0140-6736\(10\)61058-6](https://doi.org/10.1016/S0140-6736(10)61058-6)
- Sawka MN. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Medicine & Science in Sports & Exercise*. 2007;39(5):1012–20. <https://doi.org/10.1097/00005768-200002000-00012>
- Convertino VA. Blood volume: its adaptation to endurance training. *Medicine & Science in Sports & Exercise*. 1991;23(12):1338–48.
- Mairbäurl H. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Frontiers in Physiology*. 2013;4:332. <https://doi.org/10.3389/fphys.2013.00332>
- Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *American Journal of Hematology*. 2020;95(12):1599–613. <https://doi.org/10.1002/ajh.26008>
- Boykov VL, Melnikov AA. Physiological characteristics of hematological, biochemical blood parameters and sympathovagal balance in highly qualified athletes. *Human. Sport. Medicine*. 2021;21(1):7–13 (In Russ.).
- Vannucchi AM, Barbui T, Cervantes F, Harrison C, Kiladjian JJ, Kröger N, et al. ESMO Guidelines Committee. Philadelphia chromosome-negative chronic myeloproliferative neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. 2015;26(5):85–99. <https://doi.org/10.1093/annonc/mdv203>
- Koizume S, Miyagi Y. Lipid Droplets: A Key Cellular organelle associated with cancer cell survival under normoxia and hypoxia. *International Journal of Molecular Sciences*. 2016;17(9):1430. <https://doi.org/10.3390/ijms17091430>

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BIOENGINEERED HYDROGEL-BASED SCAFFOLD FOR MUSCLE REGENERATION

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Introduction. Injuries, tumor resections, and degenerative diseases can lead to extensive skeletal muscle defects, and consequently, to functional impairment and severe disability. Over the past few decades, various approaches to skeletal muscle tissue engineering have been developed. However, there remains a high demand for new effective methods and materials that promote functional regeneration, which could be used in the clinical treatment of muscle defects.

Objective. Development of a bioengineered polymer hydrogel-based scaffold for extensive skeletal muscle defects.

Materials and methods. Bioengineered scaffold samples were obtained by hydrogel cryostructuring followed by lyophilization. The microstructure and morphology of the scaffold were analyzed using a Tescan VEGA III scanning electron microscope. The mechanical properties, tensile strength, and relative elongation were analyzed using an Instron ElectroPuls E3000 testing instrument. The cytotoxicity of the scaffold was studied using fluorescence microscopy on a culture of murine fibroblasts Balb/3T3 clone A31 and NCTC clone 929 culture. The statistical significance of differences was determined using a one-way analysis of variance (ANOVA) followed by the Holm–Šidák multiple comparison test, with the critical significance level α set at 0.05.

Results. A modified scaffold was developed, representing a layered construct based on a combination of natural polymers, collagen, and sodium hyaluronate, with the addition of crosslinking agents to form layers with varying resorption rates and proteolytic degradation profiles. The scaffold samples were found to possess a multilayered porous spongy structure. The core layer (1.2 ± 0.1 mm thick) exhibited high — uniform and interconnected — porosity with nearly spherical pores ranging from 100–150 μm in size and a polymer framework wall thickness of less than 5 μm . The outer layer (300–500 μm thick) had a denser, lamellar structure with a polymer framework wall thickness of less than 2 μm and slit-shaped pores up to 200 μm in length and 50 μm in width. Compared to the core layer, significantly lower pore interconnectivity was observed, a channel structure was virtually absent, and pores were mostly isolated from one another by polymer walls. Tensile strength was 50 ± 0.5 kPa, and relative elongation was $26 \pm 6\%$. Sterilization had no effect on the strength and elongation parameters. The absence of a cytotoxic effect of the obtained construct was demonstrated: no statistically significant differences in the number of dead cells compared to the control were detected at any of the observation time points (24 and 96 h).

Conclusions. Scaffold samples with a porous structure were obtained using a combination of layer-by-layer casting/molding methods with stepwise freezing and structure control, followed by lyophilization. For the developed construct, an ethylene oxide sterilization protocol was established. The sterilization process was found to have no effect on the strength and elongation parameters. The developed methods for obtaining biocompatible constructs based on polymer hydrogels and their modification techniques will make it possible to obtain devices with a high degree of biocompatibility for enhanced tissue regeneration.

Keywords: bioengineered scaffold; collagen; sodium hyaluronate; albumin; hydrogel; cytotoxicity; mechanical properties

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БИОИНЖЕНЕРНЫЙ СКАФФОЛД НА ОСНОВЕ ГИДРОГЕЛЯ ДЛЯ РЕГЕНЕРАЦИИ МЫШЦ

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Введение. Травмы, резекции опухолей и дегенеративные заболевания могут привести к обширным дефектам скелетных мышц и, следовательно, функциональным нарушениям и тяжелой инвалидности. В последние десятилетия были разработаны различные стратегии тканевой инженерии скелетных мышц, однако по-прежнему наблюдается высокий спрос на создание новых

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высокоэффективных методов и материалов, способствующих функциональной регенерации, которые могли бы быть использованы при терапии мышечных дефектов в клинической практике.

Цель. Разработка медицинского изделия (биоинженерного скаффолда) на основе полимерного гидрогеля для заживления обширных дефектов скелетных мышц.

Материалы и методы. Образцы биоинженерного скаффолда получены путем криоструктурирования гидрогеля с последующей лиофилизацией. Микроструктуру и морфологию скаффолда анализировали при помощи сканирующего электронного микроскопа Tescan VEGA III. Анализ механических свойств, прочность на разрыв и относительное удлинение проводили при помощи испытательной машины Instron ElectroPuls E3000. Цитотоксичность скаффолда была изучена с помощью флуоресцентной микроскопии на культуре мышечных фибробластов Balb/3T3 клон A31 и культуре NCTC клон 929. Статистическую значимость отличий определяли с помощью одностороннего дисперсионного анализа ANOVA с последующим множественным сравнением Холма – Сидака, критический уровень значимости α приняли равным 0,05.

Результаты. Разработан модифицированный скаффолд, представляющий собой слоистую конструкцию на основе комбинации натуральных полимеров, коллагена и гиалуроната натрия с добавлением сшивающих агентов для формирования слоев с различными сроками резорбции и протеолитической деградации. Выявлено, что скаффолды обладали многослойной пористой губчатой структурой: средний слой (толщиной $1,2 \pm 0,1$ мм) имел крупную равномерную взаимосвязанную пористость, форма пор была близка к сферической, размер 100–150 мкм, толщина стенок полимерного каркаса менее 5 мкм. Верхний слой (толщиной 300–500 мкм) имел более плотную слоистую структуру, толщина стенок полимерного каркаса менее 2 мкм, форма пор щелевидная размером до 200 мкм в длину и 50 мкм в ширину. В сравнении с центральным слоем наблюдали значительно меньше взаимосвязанности пор, также практически отсутствовала канальная структура, а поры в основном были изолированы друг от друга стенками полимера. Прочность на разрыв составила $50 \pm 0,5$ кПа, относительное удлинение $26 \pm 6\%$; стерилизация не оказывала влияния на показатели прочности и относительное удлинение. Показано отсутствие цитотоксического действия полученной конструкции: статистически значимых различий в количестве погибших клеток относительно контрольных условий на всех сроках наблюдений (24 и 96 ч) выявлено не было.

Выводы. Пористая структура образцов скаффолда была получена с помощью комбинации методов послойной заливки / формирования с пошаговой заморозкой и контролем структуры с последующим лиофильным высушиванием. Подобран протокол стерилизации разработанной конструкции с помощью этиленоксида, а также продемонстрировано отсутствие влияния процесса стерилизации на показатели прочности и относительное удлинение. Разрабатываемые методы создания биосовместимых конструкций на основе полимерного гидрогеля и способы их модификации позволяют получать изделия с высокой степенью биосовместимости для улучшения регенерации тканей.

Ключевые слова: биоинженерный скаффолд; коллаген; гиалуронат натрия; альбумин; гидрогель; цитотоксичность; механические свойства

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Соответствие принципам этики: исследование не требовало заключения локального биоэтического комитета. В работе использовали эмбриональные мышечные фибробласты CCL 1 (NCTC клон 929) и CCL 163 (Balb/3T3 клон A31), полученные из Российской коллекции клеточных культур (ИНЦ РАН, Санкт-Петербург).

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INTRODUCTION

The development of innovative tissue-engineered constructs is a priority direction of modern regenerative medicine. Skeletal muscle regeneration is a complex process that depends on the migration of various cell types into the wound bed, signaling molecules, mechanical signals from the extracellular matrix, and its physicochemical properties. Over the past few decades, various strategies for skeletal muscle tissue engineering have been developed. However, there remains a high demand for new, more effective methods and materials capable of promoting the restoration of skeletal muscle structure and functional regeneration, which could be used in the clinical treatment of muscle defects [1].

Muscle regeneration depends on the presence of a heterogeneous population of resident muscle stem

cells (also referred to as satellite cells), the presence of interstitial cells, and the formation of blood vessels *de novo*. This process is mainly controlled by extracellular matrix proteins and associated factors (TGF- β , IL-4) [2]. The transplantation of allogeneic muscle cells is currently the clinical approach of choice for healing muscle tissue defects. However, this therapy has significant limitations due to the risk of rejection reactions, the presence of various pathologies in the donor, and an insufficient number of donor cells [3]. Furthermore, the problem of delivery and retention of cells in muscles remain unresolved, which hinders sustained regeneration necessary for adequate functional recovery [4, 5]. Therefore, the implementation of alternative strategies is required. Scaffolds based on biomaterials are a powerful tool for restoring the cellular microenvironment and ensuring the regeneration of skeletal muscle,

whose contractile strength fully corresponds to healthy tissue [5–8].

In order to induce skeletal muscle regeneration, it is necessary to mimic the native microenvironment of cells, not only by recreating the extracellular matrix through proper selection of biomaterials and methods for obtaining 3D scaffolds, but also by incorporating biologically active compounds into the composition. In terms of their micro- and nanostructure and physicochemical properties, modern scaffolds represent an artificial extracellular matrix for cells, replicating the properties of native tissue. Additionally, biologically active agents, such as proteins and growth factors, incorporated into the scaffold support cell adhesion and proliferation, inducing cells to synthesize their own extracellular matrix. To ensure mechanical, structural, physicochemical, and biological properties optimal for skeletal muscle regeneration, a number of multifunctional biomaterials have been developed. Such materials are capable of performing several important functions, such as influencing cell migration and proliferation [9], delivering drugs [10], and regulating stem cell differentiation [11].

One of the most promising types of constructs for skeletal muscle regeneration are scaffolds derived from hydrogels. Hydrogels are 3D crosslinked hydrophilic polymer matrices with a high content of water, which mimic the native aqueous environment of the body [7, 12] and structurally resemble the natural extracellular matrix. Importantly, hydrogel-based constructs can be produced in various shapes [4].

Biocompatible materials used to obtain hydrogel-based scaffolds must possess the following characteristics: degradation rates and mechanical properties corresponding to native muscle tissue; absence of toxic effects and a low risk of inflammation; and the ability to support cell migration, adhesion, and proliferation [13, 14].

A wide range of materials, both natural and synthetic, can be used for skeletal muscle regeneration. Synthetic polymers such as poly-L-lactic acid (PLA) [15, 16], poly(L-lactide-co-glycolide) (PLGA) [16], polycaprolactone [17–20], polyethylene glycol (PEG) [21–23] and their copolymers [24], as well as various polyurethanes [25–28] have found wide application for muscle tissue engineering. Among the advantages of synthetic polymers are the relative simplicity of their processing and structuring, the possibility to control mechanical properties and the release rate of biologically active compounds. However, such materials may exhibit not only low biocompatibility but also produce toxic degradation products or lack biodegradability [6, 29]. Current strategies for developing highly effective constructs for healing muscle defects are based on creating scaffolds from natural materials due to their combination of biocompatibility and biodegradability, resulting in the formation of non-toxic compounds

[7]. Components of the extracellular matrix, such as collagen [30, 31], fibrin [32, 33], hyaluronic acid [34], etc., most often serve as such materials. Polymers of natural origin, e.g., alginate [35–37], silk fibroin, and gelatin [12, 30, 38] are also widely used.

Despite the advantages of natural polymers, creating constructs on their basis is associated with numerous challenges, such as insufficient mechanical strength and elasticity, low solubility, lack of stability under physiological conditions, and difficulty in structuring the materials. To date, transplantation of muscle autografts remains the only clinically proven approach for repairing extensive skeletal muscle defects. Currently, only one material — decellularized porcine skin (XenMatrix™ AB Surgical Graft, BD, USA) — is undergoing clinical trials. Therefore, the search for new materials and the development of approaches to create effective constructs for the regeneration of skeletal muscle defects remain urgent priorities in tissue engineering.

The aim of this research was to develop a bioengineered scaffold based on a polymer hydrogel for managing extensive skeletal muscle defects.

MATERIALS AND METHODS

Bioengineered scaffold preparation

The core layer of the scaffold was formed by a mixture of 2% collagen (Belkozin Luga Plant, Russia), 1% sodium hyaluronate prepared in 0.9% NaCl (Platina, Russia), and a 10% albumin solution (Ivanovo Regional Blood Transfusion Station, Russia) in a volume ratio of 70:20:10, respectively. The middle layer consisted of 2% collagen, 1% sodium hyaluronate, and 10% albumin solution in a volume ratio of 30:35:35. The outer layer of the scaffold consisted of a 2% collagen solution, a 1% hyaluronic acid solution, and a 10% albumin solution in a volume ratio of 10:80:10, respectively. A solution comprising 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (Macklin LLC, China) and N-hydroxysuccinimide (NHS) (Sigma-Aldrich, USA) in a 4:1 ratio was used as a crosslinking agent. To form the scaffold, the biopolymer hydrogel mixture was foamed and poured into molds printed using 3D prototyping. The samples were then stepwise frozen and dried in a freeze dryer (Labconco, USA).

Sterilization

Gas low-temperature sterilization with ethylene oxide was chosen as the sterilization method, in accordance with GOST ISO 11135-2017.¹

Each scaffold sample was individually sealed in a double gas-permeable package — Tyvek film (DuPont de Nemours, Inc., Delaware, USA). Sterilization was carried out using a Steri-Vac 8XL (224 L) gas sterilizer/

¹ GOST ISO 11135-2017 "Interstate Standard. Sterilization of health-care products. Ethylene oxide. Requirements for the development, validation and routine control of a sterilization process for medical devices".

aerator (3M Health Care, St. Paul, USA). Sterilization program No. 1 according to GOST ISO 11135-2017 was used: sterilization temperature — 37 °C, sterilization exposure duration — 180 min, ethylene oxide concentration — 100%. To determine the exact concentration in the sterilizer chamber, the gas cylinder was weighed before and after sterilization. Based on the calculations, the ethylene oxide concentration inside the chamber was taken to be no less than 730.0 ± 30.0 mL/L. Aeration of the samples after ethylene oxide sterilization with sterile air was carried out for 12 h.

Scanning electron microscopy

The microstructure and morphology of the sample surface and cross-sections were studied using a Tescan VEGA III microscope (Scanning Electron Microscopy (SEM), Brno, Czech Republic), equipped with an energy dispersive spectroscopy (EDS) system (INCA Energy, Oxford Instruments, Abingdon, UK) for chemical composition analysis. The samples were pre-coated with gold using a Q150R Quorum Technologies (Lewes, UK) system. Images of the material surfaces were obtained at a pressure of 7.3×10^{-2} Pa in the column and 1.5×10^{-1} Pa in the chamber.

Mechanical properties of the obtained scaffold

To determine the tensile strength characteristics, the obtained scaffolds were used to cut dumbbell-shaped specimens with the dimensions shown in Fig. 1. The specimens were prepared simultaneously under identical conditions. Care was taken to ensure smooth surfaces without visible defects. Prior to testing, the prepared specimens were immersed in physiological saline (0.9% NaCl solution) at a temperature of 37 ± 2 °C for half an hour followed by their placement in the grips of the testing machine. During mounting, care was taken to prevent slippage and avoid damage to the specimen at the gripping points.

The tensile strength and elasticity of the obtained materials were studied using an Instron ElectroPuls E3000 testing machine (Instron, USA) at a loading rate of 5.0 mm/min, using five specimens in each series. The experiments were conducted in physiological saline (0.9% aqueous NaCl solution) at a temperature of 37 ± 2 °C. Prior to testing, the specimens were kept for half an hour in physiological saline at a temperature of 37 ± 2 °C following by their mounting in the holders. During the experiment, the specimen was stretched along its main longitudinal axis, measuring the sustained load with an error not exceeding 1% of the measured value.

The tensile strength at break was calculated using formula (1):

$$\sigma = \frac{F \times 1000}{S} \times 100\%, \quad (1)$$

where F is the tensile load at which the specimen failed (H); S is the cross-sectional area of the specimen (mm^2); σ is tensile strength at break (kPa).

An identical approach was used to study elasticity. Dumbbell-shaped specimens were also cut from the scaffold samples, with the dimensions indicated in Fig. 1. The specimens were prepared simultaneously under identical conditions following visual assessment of their integrity.

The relative elongation at break was calculated using formula (2):

$$\varepsilon = \frac{l_2^* - l_2}{l_2} \times 100\%, \quad (2)$$

where l_2 is the initial length of the narrow section of the specimen (mm); l_2^* is the length of the narrow section of the specimen at the moment of rupture (mm); ε is relative elongation at break (%).

To determine the bulk compression modulus, defined as the ratio of the stress increment to the corresponding increment in relative compressive strain, rectangular specimens (80 ± 2 mm $\times$ 10 ± 2 mm) were cut from the obtained scaffolds. The specimen thickness was 4 ± 0.5 mm. The specimens were prepared simultaneously under identical conditions. During specimen preparation, care was taken to ensure smooth surface free of visible defects.

Evaluation of the cytotoxic effect of the developed materials on CCL 1 (NCTC clone 929) and CCL 163 (Balb/3T3 clone A31) cells using fluorescence microscopy

Cytotoxicity study of the obtained scaffold samples was carried out in accordance with GOST ISO 10993-5-2011.² This method is based on determining the ratio of

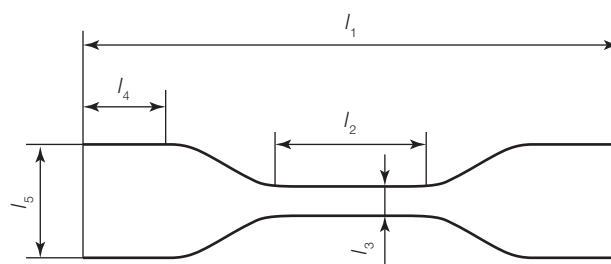


Figure prepared by the authors based on their own data

Fig. 1. Dumbbell-shaped specimen for tensile mechanical testing: l_1 — total length (60 ± 5 mm); l_2 — length of the narrow section (20 ± 0.5 mm); l_3 — width of the narrow section (10 ± 0.5 mm); l_4 — length for grip attachment (10 ± 0.5 mm), l_5 — total width (20 ± 0.2 mm); thickness of the specimen — 4 ± 0.5 mm

² GOST ISO 11135-2017 "Interstate Standard. Sterilization of health-care products. Ethylene oxide. Requirements for the development, validation and routine control of a sterilization process for medical devices".

live to dead cells cultured on the material by staining the cells with fluorescein diacetate (stains the cytoplasm of live cells green) and propidium iodide (stains the nuclei of dead cells red), using fluorescence or scanning confocal microscopy.

The cells were cultured in a mixture of DMEM/F12 media (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (HiMedia Laboratories, South America), 40 µg/mL gentamicin sulfate (Sigma-Aldrich, USA) at 37 °C under conditions of 5% CO₂ in the gas mixture. For analysis, cells were seeded into wells of a 96-well plate (SPL Life Sciences, South Korea) containing samples of the developed materials, at a concentration of 5×10^4 cells/mL. Following 24 and 96 h of incubation on the developed material, the cells were stained in L-15 medium (Sigma-Aldrich, USA) with 1% fetal bovine serum (HiMedia Laboratories, South America), containing 1 µg/mL fluorescein diacetate (Sigma-Aldrich, USA) and 2 µg/mL propidium iodide (Sigma-Aldrich, USA), for 25 min at 37 °C. Following incubation, the cells were washed twice with L-15 medium supplemented with 1% fetal bovine serum. Analysis of live and dead cells was performed using a Nikon Eclipse Ti-E microscopic station (Nikon, Japan) and a TCS SP5 scanning confocal microscope (Leica, Germany). More than 500 cells were evaluated for the analysis.

The live cells number (LCN) after incubation on the developed material was calculated as a percentage relative to the control using formula (3):

$$LCN = \frac{LCN_m}{LCN_c} \times 100\%, \quad (3)$$

where LCN_m is the live cells number after incubation on the developed material; LCN_c is the live cells number under control conditions (not incubated on the developed material).

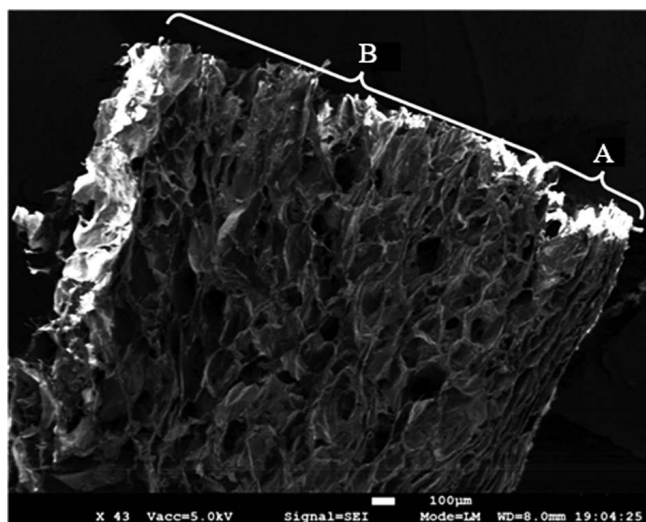


Figure prepared by the authors based on their own data

Fig. 2. Microstructure of the bioengineered scaffold sample: A — outer layer; B — core layer (SEM, magn. $\times 43$)

Statistical data processing

Statistical analysis was performed using the Python 3 (version 3.9.16) and R (version 4.3.0) programming languages in the Spyder (version 5.4.1) and RStudio (version 2023.03.0+386) software environments, respectively, employing the Pandas (version 1.5.3), NumPy (version 1.23.5), SciPy (version 1.10.0), and PMCMRplus (version 1.9.10) packages. Results were presented as the mean value and standard deviation ($M \pm SD$). Experiments were carried out in at least three replicates ($n \geq 3$). The statistical significance of differences was determined using a one-way analysis of variance (ANOVA) followed by the Holm–Šidák multiple comparison test, with the critical significance level α set at 0.05.

RESULTS AND DISCUSSION

As a result of the experiments, a porous bioengineered scaffold was obtained. This material represents a layered construct based on a combination of natural polymers, collagen, and sodium hyaluronate with the addition of EDC and NHS to form layers with different resorption rates and proteolytic degradation profiles [40]. Crosslinking agents are added to improve the mechanical strength and structural stability of collagen scaffolds upon hydration, in order to extend the possibilities of their application in certain tissues.

The porous structure of the scaffold samples was obtained using a combination of layer-by-layer casting/molding of foamed polymers followed by stepwise freezing.

The microstructure analysis using SEM established the multilayered porous spongy structure of the polymer scaffold. The core layer, 1.2 ± 0.1 mm thick, exhibits high — uniform and interconnected — porosity (Fig. 2), with pores nearly spherical in shape, 100–150 µm in size, and a polymer framework wall thickness of less than 5 µm. The outer layer, 300–500 µm thick, shows a denser lamellar structure, with a polymer framework wall thickness of less than 2 µm and slit-shaped pores up to 200 µm in length and 50 µm in width. Compared to the core layer, significantly lower pore interconnectivity was observed, a channel structure was virtually absent, and pores were mostly isolated from one another by polymer walls. This architecture of the construct was achieved through cryostructuring by stepwise freezing of the sample, which is consistent with literature data [41].

For subsequent experiments, an ethylene oxide sterilization protocol for the developed construct was selected. This method is considered optimal for polymeric implantable devices [42].

Studies were conducted to evaluate the mechanical properties of the obtained scaffold: tensile strength was 50 ± 0.5 kPa, and relative elongation was $26 \pm 6\%$. Sterilization was found to have no effect on the strength and elongation parameters (Fig. 3). It should be noted that the obtained values of mechanical strength

indicators ensure the feasibility of surgical manipulations, which is especially important for conducting further *in vivo* studies [43].

In vitro cytotoxicity testing is a critical initial step in evaluating the safety of any medical device. It enables early identification and exclusion of potentially hazardous materials, thereby minimizing risks to living organisms and reducing the number of subsequent animal trials. In the present study, assessment of the cytotoxic effect of porous bioengineered scaffold samples established the absence of their cytotoxicity (Fig. 4). No statistically significant differences in the number of dead cells relative to the control were detected at any of the observation time points (24 and 96 h). Cytotoxic components, such as degradation products and residual monomers, can induce cellular stress that may lead to

chronic inflammation and fibrosis rather than *de novo* tissue regeneration. Skeletal muscle tissue has a limited capacity for self-repair in cases of volumetric damage [44]. The absence of cytotoxicity suggests that the implant is unlikely to suppress the proliferation and migration of myoblasts and satellite cells necessary for the formation of new muscle fibers [45].

The obtained results formed the basis for an application for state registration of intellectual property, resulting in the granting of a patent for the invention “Method for obtaining a bioengineered scaffold based on a hydrogel” [46].

CONCLUSION

A porous bioengineered scaffold based on a composition of natural polymers has been developed. The porous

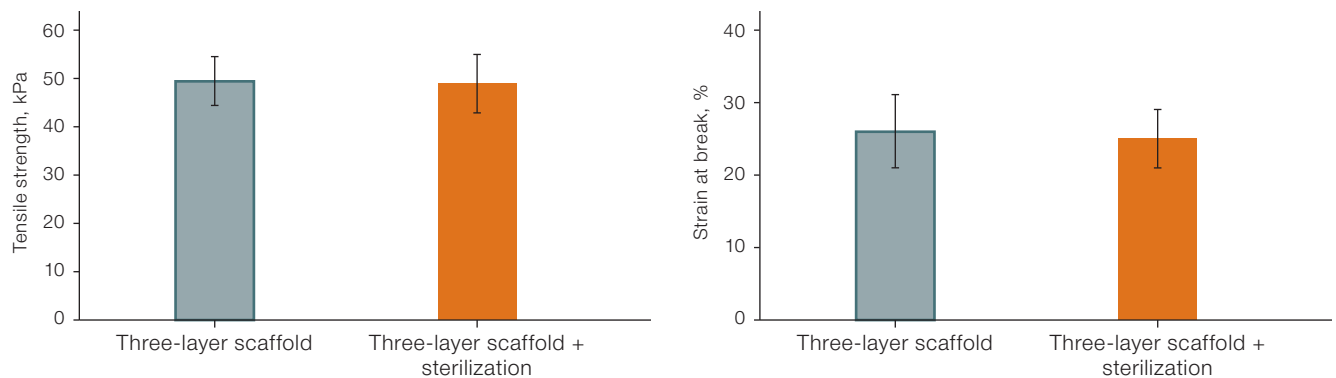


Figure prepared by the authors based on their own data

Fig. 3. Tensile strength and relative elongation of bioengineered scaffold samples

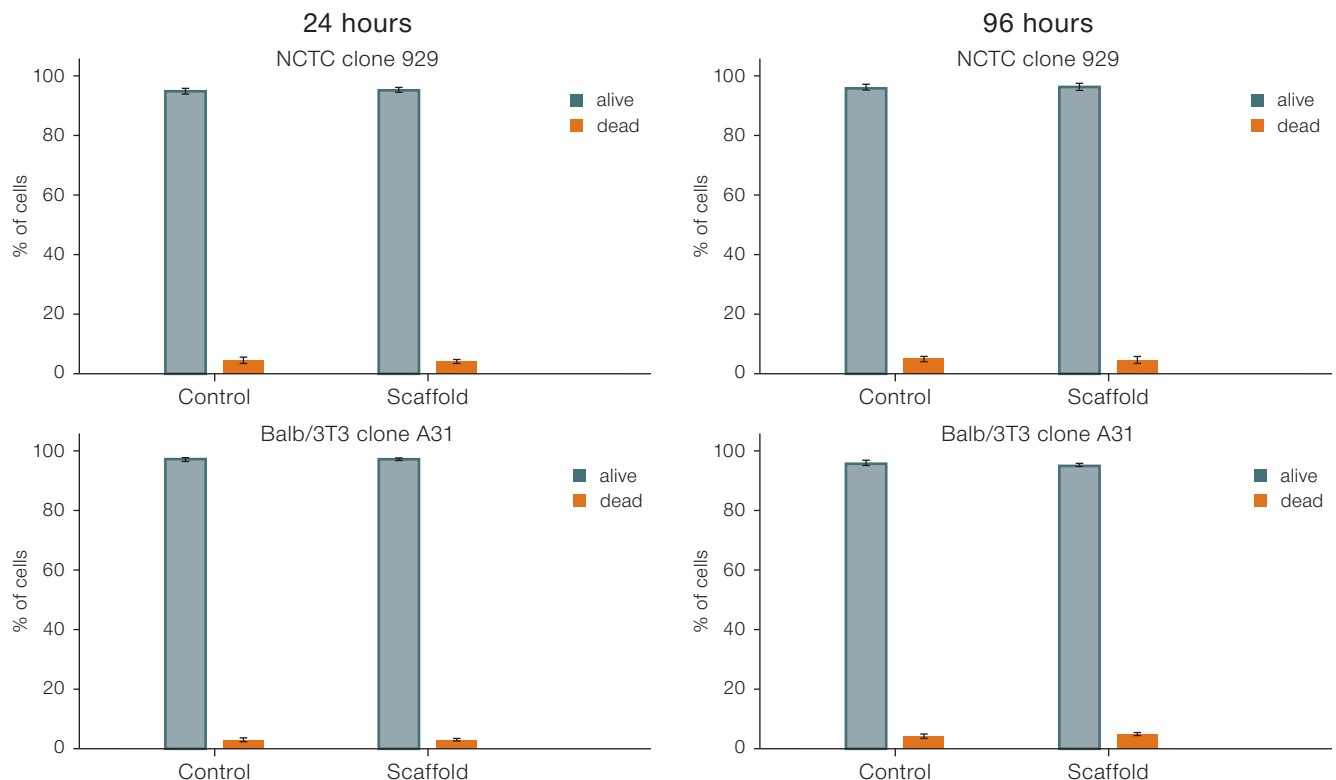


Figure prepared by the authors based on their own data

Fig. 4. Cytotoxicity assessment of the obtained bioengineered scaffold

architecture of the scaffold was achieved through a combination of layer-by-layer casting/molding methods with stepwise freezing and structure control, followed by lyophilization. An ethylene oxide sterilization protocol for the developed construct was selected. It was demonstrated that the sterilization process had no effect on strength and elongation parameters. As a result of *in vitro* tests conducted on murine fibroblast cells Balb/3T3

clone A31 and NCTC clone 929, the developed bioengineered scaffold showed no cytotoxicity. The developed methods for creating biocompatible constructs based on polymer hydrogels, along with their modification techniques, will make it possible to expand the range of medical materials available for clinical application. This will contribute to improving patients' quality of life and reducing population-level disability.

References

- Alarcin E, Bal-Öztürk A, Avci H, Ghorbanpoor H, Dogan Guzel F, Akpek A, et al. Current Strategies for the Regeneration of Skeletal Muscle Tissue. *International Journal of Molecular Sciences*. 2021;22(11):5929. <https://doi.org/10.3390/ijms22115929>
- Forcina L, Cosentino M, Musarò A. Mechanisms Regulating Muscle Regeneration: Insights into the Interrelated and Time-Dependent Phases of Tissue Healing. *Cells*. 2020;9(5):1297. <https://doi.org/10.3390/cells9051297>
- Zhou L, Ge J, Wang M, Chen M, Cheng W, Ji W, et al. Injectable muscle-adhesive antioxidant conductive photo-thermal bioactive nanomatrix for efficiently promoting full-thickness skeletal muscle regeneration. *Bioactive Materials*. 2021;6:1605–17. <https://doi.org/10.1016/j.bioactmat.2020.11.005>
- Lev R, Seliktar D. Hydrogel biomaterials and their therapeutic potential for muscle injuries and muscular dystrophies. *Interface*. 2018;15(138):20170380. <https://doi.org/10.1098/rsif.2017.0380>
- Mei X, Cheng K. Recent Development in Therapeutic Cardiac Patches. *Frontiers in Cardiovascular Medicine*. 2020;7:610364. <https://doi.org/10.3389/fcvm.2020.610364>
- Nakayama KH, Shayan M, Huang NF. Engineering Biomimetic Materials for Skeletal Muscle Repair and Regeneration. *Advanced Healthcare Materials*. 2019;8(5):1801168. <https://doi.org/10.1002/adhm.201801168>
- Fischer KM, Scott TE, Browe DP, McGaughey TA, Wood C, Wolyniak MJ, et al. Hydrogels for Skeletal Muscle Regeneration. *Regenerative Engineering and Translational Medicine*. 2021;7:353–61. <https://doi.org/10.1007/s40883-019-00146-x>
- Smoak MM, Mikos AG. Advances in biomaterials for skeletal muscle engineering and obstacles still to overcome. *Materials Today Bio*. 2020;7:100069. <https://doi.org/10.1016/j.mtbio.2020.100069>
- Basurto IM, Mora MT, Gardner GM, Christ GJ, Caliri SR. Aligned and electrically conductive 3D collagen scaffolds for skeletal muscle tissue engineering. *Biomaterials Science*. 2021;9:4040–53. <https://doi.org/10.1039/D1BM00147G>
- Cezar CA, Mooney DJ. Biomaterial-based delivery for skeletal muscle repair. *Advanced Drug Delivery Reviews*. 2015;84:188–97. <https://doi.org/10.1016/j.addr.2014.09.008>
- Xing F, Li L, Zhou C, Long C, Wu L, Lei H, et al. Regulation and Directing Stem Cell Fate by Tissue Engineering Functional Microenvironments: Scaffold Physical and Chemical Cues. *Stem Cells International*. 2019;2019:180925. <https://doi.org/10.1155/2019/2180925>
- Camci-Unal G, Annabi N, Dokmeci MR, Liao R, Khademhosseini A. Hydrogels for cardiac tissue engineering. *NPG Asia Materials*. 2014;6:e99. <https://doi.org/10.1038/am.2014.19>
- Pollet BE, Rathbone CR, Wenke JC, Guda T. Natural polymeric hydrogel evaluation for skeletal muscle tissue engineering. *Journal of Biomedical Materials Research Part B Applied Biomaterials*. 2018;106:672–9. <https://doi.org/10.1002/jbm.b.33859>
- Boso D, Maghin E, Carraro E, Giagante M, Pavan P, Piccoli M. Extracellular Matrix-Derived Hydrogels as Biomaterial for Different Skeletal Muscle Tissue Replacements. *Materials*. 2020;13(11):2483. <https://doi.org/10.3390/ma13112483>
- Rico P, Rodrigo-Navarro A, Salmerón-Sánchez M. Borax-Loaded PLLA for Promotion of Myogenic Differentiation. *Tissue Engineering Part A*. 2015;21:2662–72. <https://doi.org/10.1089/ten.tea.2015.0044>
- Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, et al. Engineering vascularized skeletal muscle tissue. *Nature Biotechnology*. 2005;23:879–84. <https://doi.org/10.1038/nbt1109>
- Wolf MT, Dearth CL, Sonnenberg SB, Lobo EG, Badyak SF. Naturally derived and synthetic scaffolds for skeletal muscle reconstruction. *Advanced Drug Delivery Reviews*. 2015;84:208–21. <https://doi.org/10.1016/j.addr.2014.08.011>
- Ostrovitov S, Salehi S, Costantini M, Suthiwanich K, Ebrahimi M, Sadeghian RB, et al. 3D Bioprinting in Skeletal Muscle Tissue Engineering. *Small*. 2019;15:1805530. <https://doi.org/10.1002/sml.201805530>
- Apsite I, Uribe JM, Posada AF, Rosenfeldt S, Salehi S, Ionov L. 4D biofabrication of skeletal muscle microtissues. *Biofabrication*. 2020;12:015016. <https://doi.org/10.1088/1758-5090/ab4ccc4>
- Kook YM, Hwang S, Kim H, Rhee KJ, Lee K, Koh WG. Cardiovascular tissue regeneration system based on multiscale scaffolds comprising double-layered hydrogels and fibers. *Scientific Reports*. 2020;10:20321. <https://doi.org/10.1038/s41598-020-77187-8>
- Salimath AS, García AJ. Biofunctional hydrogels for skeletal muscle constructs. *Journal of Tissue Engineering and Regenerative Medicine*. 2016;10:967–76. <https://doi.org/10.1002/term.1881>
- Han WM, Mohiuddin M, Anderson SE, García AJ, Jang YC. Co-delivery of Wnt7a and muscle stem cells using synthetic bioadhesive hydrogel enhances murine muscle regeneration and cell migration during engraftment. *Acta Biomaterialia*. 2019;94:243–52. <https://doi.org/10.1016/j.actbio.2019.06.025>
- Han WM, Anderson SE, Mohiuddin M, Barros D, Nakhai SA, Shin E, et al. Synthetic matrix enhances transplanted satellite cell engraftment in dystrophic and aged skeletal muscle with comorbid trauma. *Science Advances*. 2018;4:eaar4008. <https://doi.org/10.1126/sciadv.aar4008>
- Xie M, Wang L, Guo B, Wang Z, Chen YE, Ma PX. Ductile electroactive biodegradable hyperbranched polylactide copolymers enhancing myoblast differentiation. *Biomaterials*. 2015;71:158–67. <https://doi.org/10.1016/j.biomaterials.2015.08.042>
- Riboldi SA, Sampaioles M, Neuenschwander P, Cossu G, Mantero S. Electrospun degradable polyesterurethane membranes: potential scaffolds for skeletal muscle tissue engineering. *Biomaterials*. 2005;26:4606–15. <https://doi.org/10.1016/j.biomaterials.2004.11.035>
- Naureen B, Haseeb ASMA, Basirun WJ, Muhamad F. Recent advances in tissue engineering scaffolds based on polyurethane and modified polyurethane. *Materials Science and Engineering: C*. 2021;118:111228. <https://doi.org/10.1016/j.msec.2020.111228>
- Ergene E, Yagci BS, Gokyer S, Eyidogan A, Aksoy EA, Yilgor Huri P. A novel polyurethane-based biodegradable elastomer as a promising material for skeletal muscle tissue engineering. *Biomedical Materials*. 2019;14:025014. <https://doi.org/10.1088/1748-605X/ab007a>
- Jamadi ES, Ghasemi-Mobarakeh L, Morshed M, Sadeghi M, Prabhakaran MP, Ramakrishna S.

- Synthesis of polyester urethane urea and fabrication of elastomeric nanofibrous scaffolds for myocardial regeneration. *Materials Science and Engineering: C*. 2016;63:106–16.
<https://doi.org/10.1016/j.msec.2016.02.051>
29. Liu J, Saul D, Böker KO, Ernst J, Lehman W, Schilling AF. Current Methods for Skeletal Muscle Tissue Repair and Regeneration. *BioMed Research International*. 2018;2018:984879.
<https://doi.org/10.1155/2018/1984879>
 30. McLaughlin S, McNeill B, Podrebarac J, Hosoyama K, Sedlakova V, Cron G, et al. Injectable human recombinant collagen matrices limit adverse remodeling and improve cardiac function after myocardial infarction. *Nature Communications*. 2019;10:4866.
<https://doi.org/10.1038/s41467-019-12748-8>
 31. Feng M, Liu X, Hou X, Chen J, Zhang H, Song S, et al. Specific angiogenic peptide binding with injectable cardiac ECM collagen gel promotes the recovery of myocardial infarction in rat. *Journal of Biomedical Materials Research Part A*. 2020;108:1881–9.
<https://doi.org/10.1002/jbm.a.36951>
 32. Liu J, Xu HHK, Zhou H, Weir MD, Chen Q, Trotman CA. Human umbilical cord stem cell encapsulation in novel macroporous and injectable fibrin for muscle tissue engineering. *Acta Biomaterialia*. 2013;9:4688–97.
<https://doi.org/10.1016/j.actbio.2012.08.009>
 33. Melly L, Grosso A, Stanciu Pop C, Yu-Hsuan C, Nollevaux M, Schachtrup C, et al. Fibrin hydrogels promote scar formation and prevent therapeutic angiogenesis in the heart. *Journal of Tissue Engineering and Regenerative Medicine*. 2020;14:1513–23.
<https://doi.org/10.1002/term.3118>
 34. Le LV, Mohindra P, Fang Q, Sievers RE, Mkrtchjan MA, Solis C, et al. Injectable hyaluronic acid based microrods provide local micromechanical and biochemical cues to attenuate cardiac fibrosis after myocardial infarction. *Biomaterials*. 2018;169:11–21.
<https://doi.org/10.1016/j.biomaterials.2018.03.042>
 35. Stilhano RS, Madrigal JL, Wong K, Williams PA, Martin PKM, Yamaguchi FSM, et al. Injectable alginate hydrogel for enhanced spatiotemporal control of lentivector delivery in murine skeletal muscle. *Journal of Controlled Release*. 2016;237:42–9.
<https://doi.org/10.1016/j.jconrel.2016.06.047>
 36. Fang R, Tian W, Chen X. Synthesis of Injectable Alginate Hydrogels with Muscle-Derived Stem Cells for Potential Myocardial Infarction Repair. *Applied Sciences*. 2017;7:252.
<https://doi.org/10.3390/app7030252>
 37. Feng J, Wu Y, Chen W, Li J, Wang X, Chen Y, et al. Sustained release of bioactive IGF-1 from a silk fibroin microsphere-based injectable alginate hydrogel for the treatment of myocardial infarction. *Journal of Materials Chemistry B*. 2020;8:308–15.
<https://doi.org/10.1039/C9TB01971E>
 38. Theus AS, Tomov ML, Cetnar A, Lima B, Nish J, McCoy K, et al. Biomaterial approaches for cardiovascular tissue engineering. *Emergent Materials*. 2019;2:193–207.
<https://doi.org/10.1007/s42247-019-00039-3>
 39. Fischer KM, Scott TE, Browe DP, McGaughey TA, Wood C, Wolyniak MJ, et al. Hydrogels for Skeletal Muscle Regeneration. *Regenerative Engineering and Translational Medicine*. 2021;7(3):353–61.
<https://doi.org/10.1007/s40883-019-00146-x>
 40. Shepherd DV, Shepherd JH, Ghose S, Kew SJ, Cameron RE, Best SM. The process of EDC-NHS Cross-linking of reconstituted collagen fibres increases collagen fibrillar order and alignment. *APL Materials*. 2015;3(1):014902.
<https://doi.org/10.1063/1.4900887>
 41. Seifert A, Gruber J, Gbureck U, Groll J. Morphological Control of Freeze-Structured Scaffolds by Selective Temperature and Material Control in the Ice-Templating Process. *Advanced Engineering Materials*. 2022;24:2100860.
<https://doi.org/10.1002/adem.202100860>
 42. Krug N, Zarges JC, Heim HP. Influence of Ethylene Oxide and Gamma Irradiation Sterilization Processes on the Properties of Poly-L-Lactic-Acid (PLLA) Materials. *Polymers*. 2023;15(16):3461.
<https://doi.org/10.3390/polym15163461>
 43. Lipatov VA, Denisov AA, Kudryavtseva TN, Vanina AS, Russu EV, Prasolov ND. In Vitro Assessment of the Performance of Collagen-Based Polymer Matrices for Tissue Engineering. *Innovative Medicine of Kuban*. 2025;10(3):76–82 (In Russ.).
<https://doi.org/10.35401/2541-9897-2025-10-3-76-82>
 44. Forcina L, Miano C, Pelosi L, Musarò A. An Overview about the Biology of Skeletal Muscle Satellite Cells. *Current Genomics*. 2019;20(1):24–37.
<https://doi.org/10.2174/1389202920666190116094736>
 45. Geddes L, Themistou E, Burrows JF, Buchanan FJ, Carson L. Evaluation of the in vitro cytotoxicity and modulation of the inflammatory response by the bioresorbable polymers poly(D,L-lactide-co-glycolide) and poly(L-lactide-co-glycolide). *Acta Biomaterialia*. 2021;134:261–75.
<https://doi.org/10.1016/j.actbio.2021.07.049>
 46. Yudin SM, Keskinov AA, Makarov VV, Yudin VS, Bobrova MM, Luss AL, et al. *Method of producing bioengineered scaffold based on hydrogel*. Patent of Russian Federation No. 2822730; 2024 (In Russ.).
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FEATURES OF A LOCAL BIOINTERFACE FORMED UPON INTEGRATION OF A KNITTED NICKEL-TITANIUM MESH INTO STRIATED MUSCLE TISSUE

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Introduction. Injuries to skeletal muscles at various sites remain a pressing clinical problem, necessitating the development of biocompatible implants that enable full regeneration and functional restoration of muscle tissue.

Objective. Assessment of the safety and efficacy of a knitted nickel–titanium fabric following its implantation into striated muscle tissue in an *in vivo* model of mechanical loading.

Materials and methods. The study was performed on 30 Wistar rats weighing 400–500 g aged 70–90 days. An implantable material made from a low-modulus superelastic TiNi wire with a diameter of 40 µm, grade TN-10, was tested. An experimental specimen was prepared in the form of a warp-knitted tape. The animals were divided into the following groups. In group “A” ($n = 10$), the material under test was implanted into the area of a fenestrated defect of the anterior abdominal wall muscle. In group “B” ($n = 10$), the material was implanted into the area of the dissected parietal peritoneum under the left dome of the diaphragm. In group “C” ($n = 10$), the material was implanted under the muscles of the anterior neck surface. The laboratory animals were withdrawn from the experiment following 14, 30, 60, and 90 days after implantation of the metal-knit fabric. The biointerface between the metal-knit fabric and skeletal muscle tissue was assessed based on histological examination and scanning electron microscopy in the setting of a long-term experiment on laboratory animals. The research was conducted using an Axio Lab.A1 microscope, an AxioCam ERc 5s camera, and the AxioVision Rel. 4.8 software.

Results. Across all test groups, on day 14 post-implantation, a thin layer of newly formed connective tissue in the implantation area was observed. On days 30, 60, and 90 of the experiment, a trend toward denser ingrowth of collagen fibers was noted, with the formation of bundles around the NiTi wire. The formation of muscle buds and fibers was recorded on day 60. By day 90, a mature tissue regenerate in the implantation zone had been identified. This regenerate consisted of muscle fibers and the metal-knit fabric, between which fibrocytes, fibroblasts, bundles of collagen fibers, and blood vessels were located.

Conclusions. The NiTi wire-based metal-knit fabric is a safe, biocompatible material, which promotes the reparative regeneration of skeletal muscle tissue under various biomechanical conditions. This material holds promise for the surgical treatment of skeletal muscle tissue defects, thus deserving further clinical studies.

Keywords: reparative regeneration; skeletal muscle tissue; nickel–titanium alloy; metal-knit fabric; implant

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Compliance with the ethical principles: the study was conducted in compliance with the bioethical principles set forth in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The research protocol was approved by the Bioethics Committee of the Siberian State Medical University (minutes No. 732 dated 06.10.2020).

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ОСОБЕННОСТИ ЛОКАЛЬНОГО БИОИНТЕРФЕЙСА ПРИ ИНТЕГРАЦИИ ТРИКОТАЖНОЙ НИКЕЛИД-ТИТАНОВОЙ СЕТКИ В ПОПЕРЕЧНОПОЛОСАТУЮ МЫШЕЧНУЮ ТКАНЬ

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Введение. Повреждения скелетных мышц различной локализации остаются актуальной клинической проблемой, требующей разработки биосовместимых имплантов, обеспечивающих полноценную регенерацию и восстановление функции мышечной ткани.

Цель исследования. Оценка безопасности и эффективности применения трикотажного никелида титана после имплантации в поперечнополосатую мышечную ткань на *in vivo* модели механического воздействия.

Материал и методы. Исследование выполнено на 30 крысах Wistar массой 400–500 г возрастом 70–90 сут. Апробировали опытный образец имплантационного материала в виде трикотажно-вязаной ленты, выполненной из сверхэластичной TiNi проволоки марки ТН-10. Животных распределяли по группам: группа «А» ($n = 10$) — металлтрикотаж в виде вязаной ленты из низко модульной сверхэластичной никелид-титановой (TiNi) проволоки диаметром 40 мкм имплантировали в зону окончатого дефекта мышцы передней брюшной стенки; группа «Б» ($n = 10$) — имплантация образца в зону рассеченной париетальной брюшины под левым куполом диафрагмы; группа «В» ($n = 10$) — имплантация образца под мышцы передней поверхности шеи. Лабораторных животных

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выводили из эксперимента на 14, 30, 60 и 90 сут после имплантации металлтрикотаж. Биоинтерфейс металлтрикотаж и скелетной мышечной ткани оценивали на основе гистологического исследования и сканирующей электронной микроскопии в условиях хронического эксперимента на лабораторных животных. Исследования проведены с использованием микроскопа Axio Lab.A1, видеокамеры AxioCamERC 5s и программного обеспечения AxioVision Rel. 4.8.

Результаты. На 14 сут в зоне имплантации во всех группах выявлено появление тонкого слоя вновь образованной соединительной ткани. На 30, 60 и 90 сут эксперимента в исследуемых группах наблюдали тенденцию к более плотному врастанию коллагеновых волокон с формированием пучков вокруг TiNi проволоки. На 60 сут зафиксировано формирование мышечных почек и волокон. На 90 сут в зоне имплантации определялся зрелый тканевой регенерат, включающий мышечные волокна и металлтрикотаж, между которыми были расположены фиброциты, фибробласты, пучки коллагеновых волокон и кровеносные сосуды.

Выводы. Металлотрикотаж из TiNi проволоки безопасен, биосовместим и способствует репаративной регенерации скелетной мышечной ткани в различных биомеханических условиях. Материал перспективен для хирургического лечения дефектов скелетной мышечной ткани и требует дальнейших клинических исследований.

Ключевые слова: репаративная регенерация; скелетная мышечная ткань; никелид титана; металлтрикотаж; имплант

Для цитирования: Топольницкий Е.Б., Шефер Н.А., Марченко Е.С., Фомина Т.И., Гарин А.С. Особенности локального биоинтерфейса при интеграции трикотажной никелид-титановой сетки в поперечнополосатую мышечную ткань. *Экстремальная биомедицина*. 2026;28(2):250–257. <https://doi.org/10.47183/mes.2025-425>

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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 ФГБОУ ВО «Сибирский государственный медицинский университет» Минздрава России (протокол № 732 от 06.10.2020).

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INTRODUCTION

Mechanical impacts or excessive physical exertion experienced during military conflicts, emergency situations, sports events, and everyday life can lead to impaired integrity of chest or limb muscles in humans. The severity of injury determines the selection of treatment approaches and the duration of rehabilitation. A muscle can be injured at its base, tendon, muscle belly, or musculotendinous junction, leading to fiber rupture, inflammation, and impaired contractile function [1–4]. In surgical practice, iatrogenic injuries to striated muscle tissue occurring when performing surgical access are highly common. Such injuries result from the dissection of soft tissues, which inevitably compromises the integrity of the perimysium and separates the muscle fiber bundles [5, 6].

Red, intermediate, and white muscle fibers are present in all human skeletal muscles. The predominance of their certain type determines the speed of response and contraction. Red muscle fibers are characterized by a slow contraction speed and are fatigue-resistant. Conversely, white muscle fibers respond and contract rapidly, although fatigue rather rapidly. Rupturing of a small number of muscle fibers leads to the formation of muscle buds at their ends; these subsequently fuse, thereby filling the damaged area. In cases of extensive muscle defects, another mechanism of reparative regeneration after injury is involved, characterized by pronounced proliferation of myosatellite cells, their migration into the defect area, and the formation of new myotubes. This requires specific conditions, such as

the absence of fibroblasts in the regeneration area and preserved motor innervation of the muscle fibers. Otherwise, a dense connective tissue scar forms [7–9]. In order to prevent fibrosis and scarring in the area of surgical trauma, which lead to impaired muscle function, biological and synthetic implants can be used. The development and clinical implementation of such implants are actively pursued in scientific centers worldwide, including Russia. There are reports on the use of porous and mesh materials made from metal alloys, hyaluronic acid-based hydrogel, sponge biomaterial, polypropylene, polytetrafluoroethylene (Gore-Tex), prolene, Parietex Composite, etc. [10–12].

Nickel–titanium-based alloys (NiTi) are increasingly attracting interest in the fields of traumatology and orthopedics, reconstructive thoracoabdominal surgery, and regenerative medicine. This interest is primarily driven by the biocompatibility of NiTi-based implants. Previous studies have shown that implants made of NiTi wire-based metal-knit fabric possess not only sufficient elasticity to conform to the contours of biological tissues, but also high resistance to long-term cyclic loading, while also facilitating the formation of a strong, unified tissue regenerate in the damaged area [13–15]. Morphological changes associated with the use of NiTi implants for integration into skeletal muscle tissue at various locations with differing physical loads have not been previously described.

The aim of this study was to assess the safety and efficacy of a knitted nickel-titanium alloy following its implantation into striated muscle tissue under an *in vivo* model of mechanical loading.

MATERIALS AND METHODS

The study was conducted at the Central Research Laboratory of the Siberian State Medical University using 30 certified male Wistar rats weighing 400–500 g and aged 70–90 days. The animals were obtained from the Department of Experimental Biological Models of the Goldberg Research Institute of Pharmacology and Regenerative Medicine (Tomsk National Research Medical Center). The animals were housed under standard vivarium conditions.¹ Prior to the experiment, the animals underwent a two-week quarantine in the vivarium in compliance with established housing requirements. A 12-h light-dark cycle was maintained, and food and water were provided *ad libitum*. No more than two animals were housed per cage. Body weight was recorded for all animals in each group before the surgical procedure and again at the end of the experiment prior to euthanasia.

During the experiment, a prototype implantable material in the form of a warp-knitted tape made of superelastic NiTi wire grade TN-10 was tested (Fig. 1).

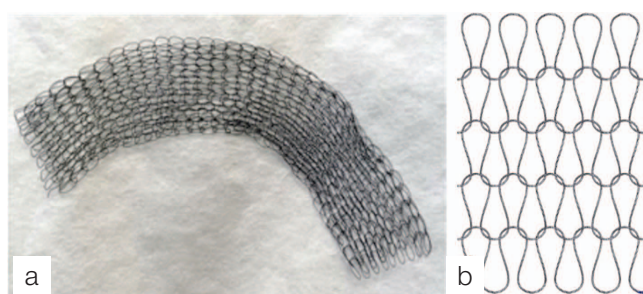


Photo taken by the authors

Fig. 1. Nickel-titanium alloy wire metal-knit fabric: a — general view of the two-layer tape; b — geometric model of a single-layer specimen

The implant was manufactured at the Laboratory of Superelastic Biointerfaces, National Research Tomsk State University.

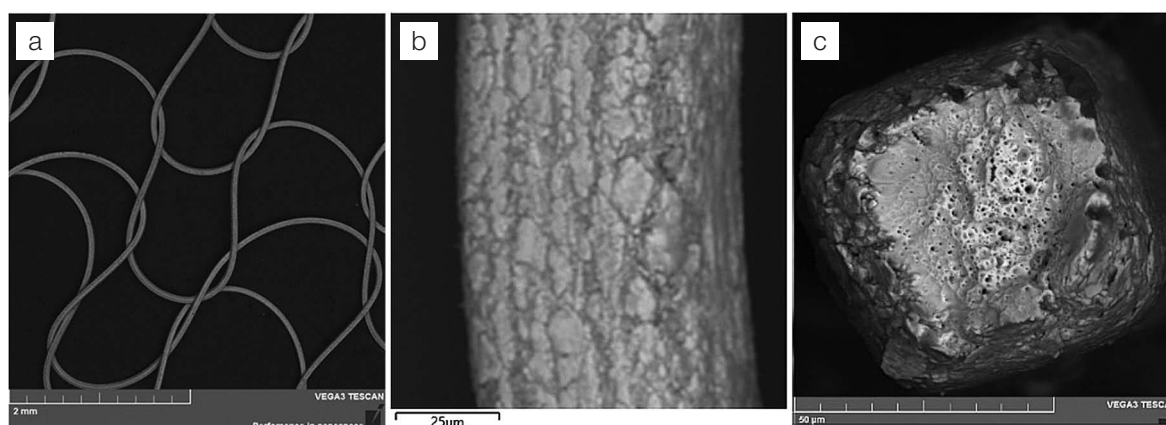
The metal-knit tape is a mesh structure made of biologically inert, corrosion-resistant NiTi wire with a diameter of 40 μm and mesh sizes ranging 0.3–3.5 mm (Fig. 2). The NiTi-based wire is a composite material characterized by the presence of an outer rough layer with a thickness of up to 1 μm .

A warp-knitted structure of the metal-knit fabric ensured non-unraveling and non-fraying edges, while the specific intertwining imparted to the fabric not only dimensional stability but also limited, uniform stretchability in all directions. To form a knitted sleeve and tape, thin NiTi wire was knitted on a Rishikesh Wire circular knitting machine (Rishikesh Electromatic Pvt. Ltd., India). The circular knitting created a closed tubular structure with a regular loop pattern, which facilitated subsequent technological transformation and ensured isotropic mechanical properties in the plane of the fabric. After knitting, the preform underwent a stabilizing anneal. To shape the metal-knit fabric into a tape, the metal-knit sleeve was subjected to hot rolling at a temperature of 500°C, forming a symmetrical two-layer structure with a predetermined porosity and cell topology, which played a key role in its functional properties.

The perioperative management of the animals in the groups did not differ, except for the area of surgical intervention.

The animals were divided into three groups:

- In group “A” ($n = 10$), the NiTi metal-knit fabric was implanted into a thoracoabdominal defect adjacent to the muscles of the anterior abdominal wall;
- In group “B” ($n = 10$), the NiTi metal-knit fabric was implanted adjacent to the dissected parietal peritoneum under the left dome of the diaphragm;



Photos taken by the authors

Fig. 2. Spatial and morphological structure of a nickel-titanium alloy wire metal-knit fabric: a — warp-knitted structure of a single-layer specimen under scanning electron microscopy (magn. $\times 50$); b — rough surface (magn. $\times 2500$); c — cross-section of the NiTi wire under scanning electron microscopy (magn. $\times 2500$)

¹ GOST 33215-2014. Guidelines for the Housing and Care of Laboratory Animals. Rules for Facilities and Procedural Organization. Moscow: Standartinform; 2019.

- In group “C” ($n = 10$), the NiTi metal-knit fabric was implanted in the pretracheal space, beneath the muscles of the anterior neck surface.

The implants were placed in skeletal muscles differing in their level of natural physiological load and the nature of contractile activity, without applying any additional external loads on the animals. This choice of implantation sites was dictated by the objective of modeling different physiological biomechanical conditions for the implant within a single experimental series.

Laboratory animals, under zolazepam-xylazine anesthesia, were secured to an operating table; the surgical area was shaved and treated with an antiseptic agent.

In group “A”, a midline laparotomy was performed, and the xiphoid process along with a musculofascial portion of the anterior abdominal wall were resected in an extrapleural manner. The post-resection thoracoabdominal defect was replaced with a metal-knit fabric tape, which was fixed along its perimeter with a continuous suture using a 4/0 polypropylene atraumatic suture material. In group “B”, following laparotomy and incision of the parietal peritoneum under the left dome of the diaphragm, the tape was placed without additional fixation. In group “C”, after an incision was made along the anterior surface of the neck and the muscles were retracted, the metal-knit fabric was placed pretracheally, beneath the deep muscles. The surgical wound was closed completely with a 4/0 Vicryl® atraumatic suture material.

After the surgical intervention, all animals were housed in individual cages (VELAZ, Czech Republic). To assess the general condition of the animals, daily clinical examination data were recorded throughout the entire observation period. The parameters evaluated included the nature of excretion, the state of the fur, skin and mucous membranes, appetite, respiration, mobility, neuromuscular excitability, gait, aggression, miosis/mydriasis/exophthalmos, etc. (SOP: CPS-SV-008). During the postoperative period, for five days, each animal received preventive antimicrobial therapy with Enrofloxacin (API SAN LLC, Russia) *per os* via drinking water at a dose of 15 mg/kg bw; the drug was pre-dissolved in 200 mL of drinking water. For analgesia during the first two days after implantation, the animals received Ketoprofen (VIK — Animal Health, Belarus) subcutaneously at a dosage of 5 mg/kg bw once daily.

Laboratory animals were euthanized following 14, 30, 60, and 90 days after implantation of the metal-knit fabric via carbon dioxide inhalation in accordance with the Standard Operating Procedure (SOP: CPS-PDI-023). The biological specimen was excised with surrounding tissues for macroscopic analysis. For histological examination, fragments of striated muscle tissue and surrounding connective tissue were collected directly from the implantation site, as well as from the visually unaltered muscle tissue at a distance of no less than 10 mm from the implant edge, which served as an internal control. The biological samples, comprising the

tissue regenerate (striated muscle tissue with adjacent connective tissue) from the implantation site, were fixed in 10% neutral buffered formalin. Upon completion of fixation, the regenerate was precisely separated along its boundary with the mesh metal-knit implant, since metal wire is not amenable to standard histological processing and sectioning. The remaining tissue was dehydrated, embedded in paraffin, sectioned at 5 μ m thickness, and stained with hematoxylin and eosin and Van Gieson's stain (for connective tissue).

For morphological examination, an Axio Lab.A1 microscope (Carl Zeiss Microscopy GmbH, Germany) was used in conjunction with an AxioCam ERc 5s video camera (Carl Zeiss Microscopy GmbH, Germany) and AxioVision Rel. 4.8 software (Carl Zeiss Microscopy GmbH, Germany). The analysis of tissue integration features with the mesh structure of the metal-knit fabric was performed using a Tescan Mira analytical scanning electron microscope (Brno, Czech Republic). After preliminary fixation in a 10% solution of neutral buffered formalin, tissue fragments containing the implanted material samples were isolated. Following freeze-drying, the samples were mounted on conductive carbon tape in the chamber of the scanning electron microscope.

RESULTS

In all laboratory animals, the postoperative wounds healed by primary intention. At day 14 and subsequent time points, no visual deformation of the anatomical structures adjacent to the implant was recorded in groups “A” and “C”. During macroscopic examination at autopsy, a trend toward denser adhesion of the implant to the adjacent muscle tissues was observed over the experimental period from the moment of implantation in groups “A” and “B”.

On day 14, in all groups, a layer of delicate connective tissue between the implant and the muscle structures was observed. In group “C”, on days 14 and 30, the metal-knit fabric and the neck muscles could be easily separated from each other due to the absence of dense adhesion between them. At all time points in the study groups, migration of the mesh implant was not observed. At later time points in group “B”, the tissue regenerate in the implant area had limited mobility due to denser adhesion; however, this did not generally restrict diaphragmatic motility. In group “C”, on days 60 and 90, the metal-knit fabric was more densely adherent to the neck muscles compared to earlier time points in the study.

Histological examination of biological samples from groups “A” and “B” on day 14 post-implantation revealed the formation of a layer of young connective tissue with small newly formed blood vessels and collagen fibers between the metal-knit fabric and the striated muscle fibers (Fig. 3). At the same time point, all

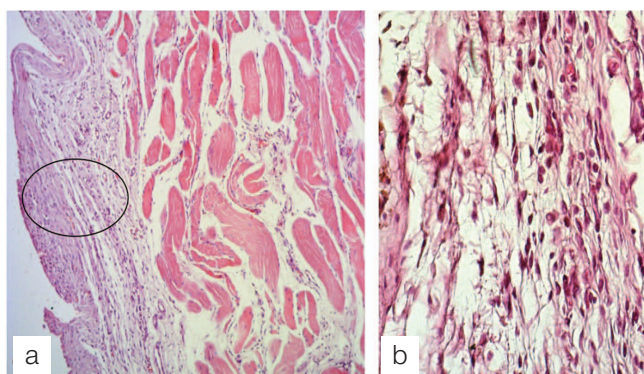
groups predominantly exhibited lympho-macrophagic inflammatory infiltration, along with minor edema and isolated neutrophils in the implant area. Lymphocytes, macrophages, and fibroblasts predominated among the cellular elements. However, in group "C" on day 14, the formation of loose, immature connective tissue on the surface of the implant was noted.

On day 30, in the implantation site in all groups, more mature connective tissue was observed, characterized by an increased number of collagen fibers and the presence of isolated, newly formed bundles composed therefrom. Histological examination of samples at day 60 post-implantation of the NiTi metal-knit fabric revealed that the collagen fibers were more structured and formed bundles (Fig. 4), with fibrocytes predominating in the cellular composition, fibroblasts being

less common, and isolated neutrophils being present. In all study groups, at day 60 post-implantation, muscle buds — a structural sign of striated muscle tissue regeneration — were detected at the sites of muscle fiber transection (Fig. 5).

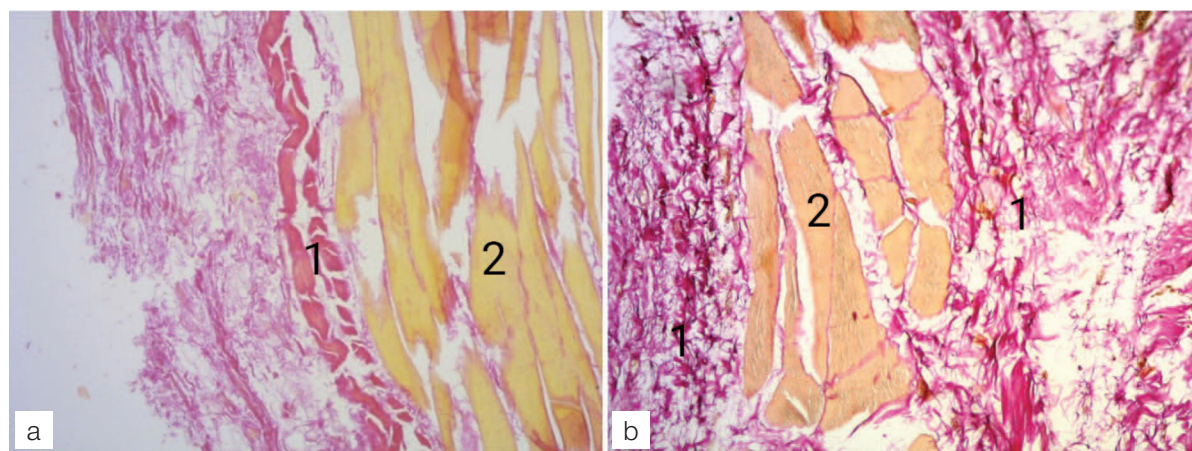
By day 90 post-implantation, histological examination in groups "A" and "B" revealed the formation of a tissue regenerate consisting of muscle fibers and mature connective tissue components. The regenerate structure contained cellular elements represented by fibrocytes and fibroblasts, exhibiting an organized, predominantly unidirectional orientation of collagen fibers. Furthermore, pronounced vascularization was observed, indicating the presence of a developing network of blood vessels that provide the regenerating tissue with the necessary metabolic conditions for further restoration and functional integration.

Examination using scanning electron microscopy on day 14 post-implantation in all groups recorded the initial formation of connective tissue on the implant surface and within the interstices of the NiTi metal-knit fabric wire (Fig. 6a). By day 30, collagen fibers and fibroblasts had been completely filled the pores of the two-layer implant in isolated areas, promoting material integration with the surrounding tissue and ensuring mechanical stability (Fig. 6b). At later time points, on days 60 and 90, dense encasement of the wire by collagen fibers was observed in the groups, with these fibers forming bundles oriented in mutually perpendicular planes. This led to the creation of a distinctive structural mesh, providing optimal conditions for implant fixation and biocompatibility (Fig. 6c). The formation of collagen fiber bundles creating various types of interweaving between the muscles of the anterior abdominal wall was noted, accompanied by a pronounced cellular ridge along the surface and at the intersections of the NiTi wire. The density of these structures progressively increased toward the later observation time points.



Photos taken by the authors

Fig. 3. Tissue regenerate on day 14 post-implantation: a — a layer of granulation tissue is visualized between the metal-knit fabric (element removed during slide preparation) and the striated muscle fibers of the abdominal wall (outlined) (magn. $\times 150$); b — a fragment of the same granulation tissue area at higher magnification: fine collagen fibers, cellular elements, and newly formed blood vessels (magn. $\times 400$)



Photos taken by the authors

Fig. 4. Proliferation of collagen fibers (1) between the muscle fibers (2) of the abdominal wall at various time points following NiTi metal-knit fabric implantation: a — observation period 30 days; b — observation period 60 days; staining with picrofuchsin according to Van Gieson (magn. $\times 150$)

DISCUSSION

During the process of reparative regeneration of skeletal muscle tissue following traumatic injury, the formation of a connective tissue scar is inevitable. This scar partially or completely replaces the defect, leading to motor dysfunction of the muscle and clinically manifesting impairment of mobility in the corresponding anatomical region. The mechanisms of reparative regeneration of muscle tissue are still under investigation and remain a relevant area of scientific research [4, 16–18]. Despite significant progress in elucidating the molecular and cellular processes regulating the restoration of striated muscle tissue, many aspects, including the role of the extracellular matrix and the interaction of various cell populations, require further detailed study [19–21].

A wide range of biomaterials and tissue-engineered constructs have been proposed for reconstructing extensive post-resection soft tissue defects, reflecting the ongoing search for an optimal solution that accounts for individual anatomical and functional requirements. Key criteria for the effective application of such materials include biocompatibility to ensure adequate integration with the surrounding stroma without immunogenic reactions and mechanical strength under cyclic multi-axial loads to prevent deformation and postoperative complications. These characteristics are essential for anatomical and physiological restoration and long-term functional rehabilitation of the damaged area [22, 23].

The present study established that the fundamental biological processes of regeneration, including granulation tissue formation, neoangiogenesis, fibroblast proliferation, and subsequent organization of the collagen matrix, exhibited similar morphological features regardless of the location of the tissue regenerate. This indicates the versatility of restoration mechanisms under the conditions of metal-knit scaffold implantation [24–26]. Previous studies, including comparisons with polypropylene implants, have shown that the formation of a vascularized collagen regenerate around the implant

is a key biological phenomenon determining successful material integration and restoration of the supporting function of soft tissues [9, 13, 27–30].

When using a NiTi metal-knit fabric as an implant in muscle tissues, the absence of a pronounced inflammatory reaction and a reliable, stable fixation of the implanted element without migration were noted. Long-term implantation of the metal-knit fabric promoted improved integration of the artificial material with biological tissues, further confirming its high level of biocompatibility. The NiTi metal-knit implant served as a scaffold for the formation and ingrowth of a connective tissue regenerate, creating a complete anatomical structure. The interaction between the mesh implant made of NiTi wire and the collagen fibers within the regenerate area ensured the restoration of anatomical and physiological integrity,

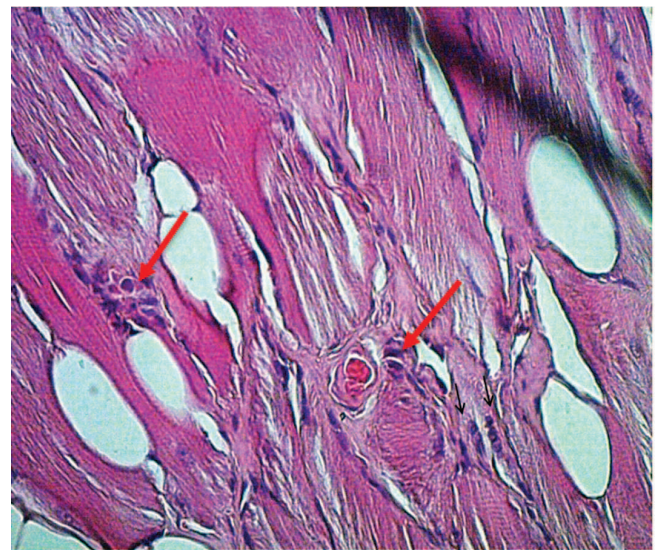
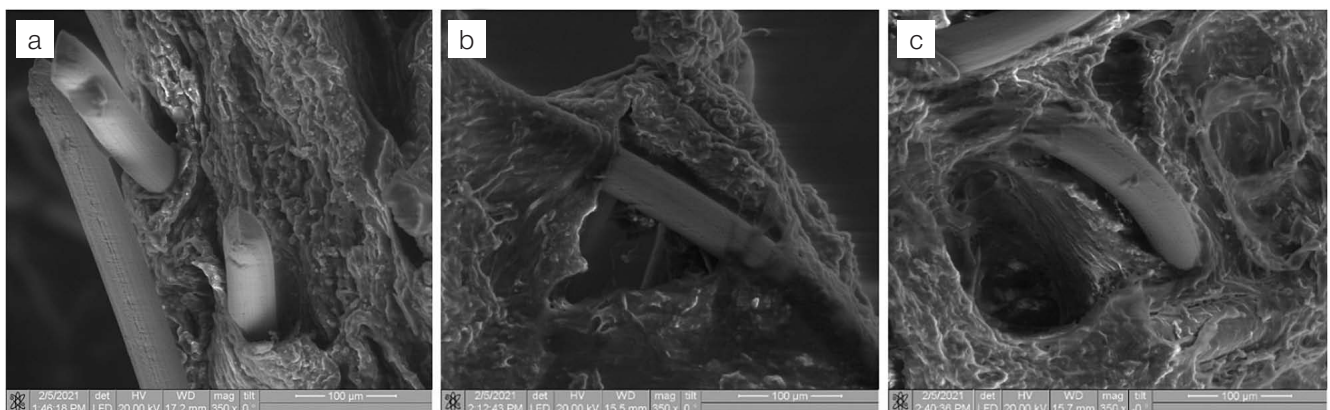


Photo taken by the authors

Fig. 5. Regeneration of skeletal muscle fibers in group “A” at 60 days post-implantation of NiTi metal-knit fabric: muscle buds are indicated by red arrows; hematoxylin and eosin staining (magn. $\times 600$)



Photos taken by the authors

Fig. 6. Tissue regenerate in group “A” at various time points post-implantation: a — day 14; b — day 30; c — day 60 (magn. $\times 350$)

which was a key factor in the regeneration and functional integrity of the muscle tissue.

The data obtained indicate that the integration of the implant into muscles with different baseline functional loads was accompanied by the formation of a unified tissue regenerate without clinically significant restriction of mobility or signs of rigidity in the corresponding muscle groups. This allows us to consider the formed scaffold to be biomechanically and functionally adequate to the recipient tissues under different physiological muscle operating conditions. The mesh NiTi material implant, after replacing the soft tissue defect, functioned as a mobile reinforcing structure, functionally analogous to the tendinous part of a muscle. The use of a metal-knit fabric with a filament diameter of 40 μm optimized the combination of mechanical strength and biocompatibility, minimizing the risk of postoperative complications and the formation of dense connective tissue scars. The presumed physiological load on the muscle group of a specific anatomical area was successfully transferred through the NiTi-based material, confirming its long-term ability to support the motor function of the muscle structure. It was additionally noted that such integration and interaction of the implant with the tissue contributed to the preservation of microcirculation and prevented

the development of coarse fibrosis, which is an essential condition for sustainable functional restoration.

CONCLUSION

The investigated metal-knit fabric based on thin NiTi wire was shown to be promising as a biomaterial for reconstructive surgery in addressing extensive skeletal muscle defects. The combination of biocompatibility, mechanical stability under dynamic loads, and pronounced capacity for tissue integration provides reliable functional support to the muscular framework, promotes anatomical-physiological restoration, and allows for the minimization of excessive fibrosis. Overall, this forms a basis for an optimal clinical outcome.

Further research should be focused on a comprehensive assessment of the long-term biomechanical characteristics and clinical efficacy of metal-knit fabric implants in various traumatic and surgical scenarios. This must include a mandatory quantitative comparative analysis of the cellular and matrix components of the regenerate. Such an approach will allow for the formation of a reliable evidence base to facilitate their broad adoption and targeted refinement in clinical practice.

References

1. Gao Y, Zhang Z, Yao Y, Zhang J, Li X, Yang K, et al. Engineering of tissue in microphysiological systems demonstrated by modelling skeletal muscle. *Regenerative Biomaterials*. 2025;12:rbaf059. <https://doi.org/10.1093/rb/rbaf059>
2. Genovese EA, Calvi M, Mazzoni S, Genesio L, Lamantea S, Vincenzo Z, et al. Proposed modified classification system of the Munich Consensus Statement. Can the area of haemorrhagic effusion in muscle injuries be the dividing line between mild (3A) and moderate (3B) injuries? *Polish Journal of Radiology*. 2025;90:e347–55. <https://doi.org/10.5114/pjr/203993>
3. Qi L, Zhang F, Wang K, Chen B, Li X, Xu J, et al. Advancements in skeletal muscle tissue engineering: strategies for repair and regeneration of skeletal muscle beyond self-repair. *Regenerative Biomaterials*. 2025;12:rbaf050. <https://doi.org/10.1093/rb/rbaf050>
4. Palmeri S, Scavone A, Anzà M, Gregorace E, Vecchiato M, Bordalo M, et al. Diagnostic ultrasound of muscle injuries: what the sports medicine clinician should know. *British Journal of Sports Medicine*. 2025;59(24):bjsports-2025-110255. <https://doi.org/10.1136/bjsports-2025-110255>
5. Sletov AA, Panchenko DV, Zhidovinov AV, Loktionova MV, Vintaev VV. Comparative analysis of the functionalism of neck anatomical structures during their delicate and aggressive dissection. *Russian Journal of Operative Surgery and Clinical Anatomy*. 2018;2(3):32–7 (In Russ.). <https://doi.org/10.17116/operhirurg2018203132>
6. Moreno-Justicia R, Van der Stede T, Stocks B, Laitila J, Seaborne RA, Van der Loock A, et al. Human skeletal muscle fiber heterogeneity beyond myosin heavy chains. *Nature Communications*. 2025;16:1764. <https://doi.org/10.1038/s41467-025-56896-6>
7. Namjoo AR, Ahrbekoh FN, Saghati S, Amini H, Saadatlou MAE, Rahbarghazi R. Tissue engineering modalities in skeletal muscles: focus on angiogenesis and immunomodulation properties. *Stem Cell Research and Therapy*. 2023;14(1):90. <https://doi.org/10.1186/s13287-023-03310-x>
8. Darkes-Burkey C, Liu X, Slyker L, Mulderrig J, Pan W, Giannelis EP, et al. Simple synthesis of soft, tough, and cytocompatible bio-hybrid composites. *PNAS*. 2022;119(28):e2116675119. <https://doi.org/10.1073/pnas.2116675119>
9. Kozan NG, Joshi M, Sicherer ST, Grasman JM. Porous biomaterial scaffolds for skeletal muscle tissue engineering. *Frontiers in Bioengineering and Biotechnology*. 2023;11:1245897. <https://doi.org/10.3389/fbioe.2023.1245897>
10. Lebedeva AI, Muslimov SA, Vagapova VSh, Shcherbakov DA. Morphological aspects of the regeneration of skeletal muscle tissue induced by allogeneic biomaterial. *Practical Medicine*. 2019;17(1):98–102 (In Russ.). <https://doi.org/10.32000/2072-1757-2019-1-98-102>
11. Kase N, Kitagawa Y, Ikenaka A, Niwa A, Saito MK. A concise *in vitro* model for evaluating interactions between macrophage and skeletal muscle cells during muscle regeneration. *Frontiers in Cell and Development Biology*. 2023;11:1022081. <https://doi.org/10.3389/fcell.2023.1022081>
12. Ge X, Jin Y, He J, Jia Z, Liu Y, Xu Y. Extracellular matrix in skeletal muscle injury and atrophy: mechanisms and therapeutic implications. *Journal of Orthopaedic Translation*. 2025;52:404–18. <https://doi.org/10.1016/j.jot.2025.03.004>
13. Topolnitskiy EB, Dambaev GTs, Gunther VE. Replacement of post-resection chest wall defects with a tissue implant based on nanostructured nickelide-titanium thread. *Pirogov Russian Journal of Surgery*. 2011;(10):47–53 (In Russ.). EDN: [PIQJXP](https://doi.org/10.1016/j.jot.2025.03.004)
14. Topolnitskiy EB, Shefer NA, Marchenko ES, Chekalkin TL, Yasenchuk YuF. Chest wall resection for neoplasms with reconstruction using titanium-nickelide implants. *P.A. Herzen*

- Journal of Oncology. 2021;10(2):22–8 (In Russ.).
<https://doi.org/10.17116/onkolog20211002122>
15. Topolnitskiy E, Chekalkin T, Marchenko E, Yasenchuk Y. Repair of huge thoracic defect combined with hernia after multimodality treatment of breast cancer. *Respiratory Medicine Case Reports*. 2021;34:101558.
<https://doi.org/10.1016/j.rmcr.2021.101558>
 16. Cemazar M, Jurdana M. Radiation-induced impairment of skeletal muscle regeneration. *Radiology and Oncology*. 2025;59(3):293–300.
<https://doi.org/10.2478/raon-2025-0048>
 17. Fan T, Jia M, Liu H, Gao Z, Huang W, Liu W, et al. Engineering strategies for the construction of oriented and functional skeletal muscle tissues. *Biofabrication*. 2025;17:022013.
<https://doi.org/10.1088/1758-5090/adbfc2>
 18. Yang W, Hu P. Skeletal muscle regeneration is modulated by inflammation. *Journal of Orthopaedic Translation*. 2018;13:25–32.
<https://doi.org/10.1016/j.jot.2018.01.002>
 19. Hebda E, Pielichowski K. Biomimetic Polyurethanes in Tissue Engineering. *Biomimetics (Basel)*. 2025;10(3):184.
<https://doi.org/10.3390/biomimetics10030184>
 20. Ahmad K, Shaikh S, Chun HJ, Ali S, Lim JH, Ahmad SS, et al. Extracellular matrix: the critical contributor to skeletal muscle regeneration—a comprehensive review. *Inflammation and Regeneration*. 2023;43(1):58.
<https://doi.org/10.1186/s41232-023-00308-z>
 21. Pillai Babu D, Sreena R, Brenner K, Nathanael AJ, Kishore V. Progress in skeletal muscle tissue engineering: advancing from 3D to 4D bioprinting. *Progress in Biomedical Engineering*. 2025;8:012003.
<https://doi.org/10.1088/2516-1091/ae1e47>
 22. Ahmad SS, Ahmad K, Lim JH, Shaikh S, Lee EJ, Choi I. Therapeutic applications of biological macromolecules and scaffolds for skeletal muscle regeneration: A review. *International Journal of Biological Macromolecules*. 2024;267(Pt 2):131411.
<https://doi.org/10.1016/j.ijbiomac.2024.131411>
 23. Cordelle MZ, Snelling SJB, Mouthuy PA. Skeletal Muscle Tissue Engineering: From Tissue Regeneration to Biorobotics. *Cyborg and Bionic Systems*. 2025;6:0279.
<https://doi.org/10.34133/cbsystems.0279>
 24. Qiu M, Li Y, Wang Q, Jian X, Huang J, Gui W, et al. Cellular Senescence in the Regenerative Niche Hampers Skeletal Muscle Repair. *Aging and Disease*. 2025;17(1):349–66.
<https://doi.org/10.14336/AD.2024.1501>
 25. Sicherer ST, Haque N, Parikh Y, Grasman JM. Current Methodologies for Inducing Aligned Myofibers in Tissue Constructs for Skeletal Muscle Tissue Regeneration. *Advances in Wound Care*. 2025;14(2):114–31.
<https://doi.org/10.1089/wound.2024.0111>
 26. Wang L, Chang L, Ma Y, Jia Y, Gao B, Cui W, et al. Mechanomedicine for Addressing Skeletal Muscle Insulin Resistance. *Endocrine Reviews*. 2025;46(5):652–69.
<https://doi.org/10.1210/endrev/bnaf012>
 27. Girotti PNC, Bianchi F. Chest wall reconstruction, prosthesis and allografts: a narrative review. *Journal of Thoracic Disease*. 2023;15(12):7077–87.
<https://doi.org/10.21037/jtd-23-650>
 28. Yasenchuk YF, Marchenko ES, Gunter SV, Baigonakova GA, Kokorev OV, Volinsky AA, et al. Softening effects in biological tissues and NiTi knitwear during cyclic loading. *Materials*. 2021;14(21):6256.
<https://doi.org/10.3390/ma14216256>
 29. Queeno SR, Sterner KN, O'Neill MC. Meta-analysis data of skeletal muscle slow fiber content across mammalian species. *Data in Brief*. 2023;50:109520.
<https://doi.org/10.1016/j.dib.2023.109520>
 30. Topolnitskiy EB, Shefer NA, Marchenko ES, Fomina TI, Mikhed RA, Tsydenova AN, et al. Features of the integration of two-layer metal knitwear made of titanium nickelide during the replacement of a thoracoabdominal defect in the experiment. *Acta Biomedica Scientifica*. 2023;8(2):244–53 (In Russ.).
<https://doi.org/10.29413/ABS.2023-8.2.24>

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PROSPECTS FOR DEVELOPING GLIOBLASTOMA THERAPY USING REDOX-TARGETING DRUGS: A NARRATIVE LITERATURE REVIEW

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Introduction. Glioblastoma is the most common primary malignant brain tumor in adults. Despite modern treatment approaches involving surgical tumor resection followed by radiation and chemotherapy, the disease is typically associated with an unfavorable prognosis with the median survival of patients after diagnosis of about 14.6 months. This is largely attributable to the high chemoresistance of glioblastoma to therapy, determined, among other reasons, by its resistance to oxidative stress.

Objective. Generalization of data on redox-dependent mechanisms of glioblastoma chemoresistance, as well as an analysis of the prospects for using drugs that destabilize redox homeostasis in glioblastoma therapy.

Discussion. In response to therapy, tumor cells activate antioxidant systems, thereby retaining the release of reactive oxygen species induced by chemotherapeutic agents, stabilizing intracellular redox homeostasis, and preventing the development of oxidative stress. In this regard, the use of compounds that enhance the generation of intracellular reactive oxygen species or suppress the activity of key components of antioxidant defense appears to be a promising approach for sensitizing tumors to therapy. A number of drugs based on such compounds, either as monotherapy or in combination with other approaches, have shown efficacy in preclinical trials and demonstrated effectiveness in treating patients with glioblastoma during clinical studies.

Conclusions. The search for more selective inhibitors of antioxidant systems, optimization of their delivery to the tumor, and patient stratification based on molecular-genetic and biochemical markers of tumor redox homeostasis could increase the effectiveness of glioblastoma therapy.

Keywords: glioblastoma; chemotherapy; oxidative stress; antioxidant systems; therapy resistance

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ПЕРСПЕКТИВЫ РАЗРАБОТКИ ТЕРАПИИ ГЛИОБЛАСТОМЫ С ПРИМЕНЕНИЕМ РЕДОКС-ТАРГЕТИРУЮЩИХ ПРЕПАРАТОВ: ОПИСАТЕЛЬНЫЙ ОБЗОР

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Введение. Глиобластома является наиболее распространенной первичной злокачественной опухолью головного мозга у взрослых. Это заболевание, как правило, имеет неблагоприятный прогноз: медианная продолжительность жизни пациентов после постановки диагноза — 14,6 мес., несмотря на современное лечение, включающее в себя хирургическое удаление опухоли с последующей лучевой и химиотерапией. Во многом это обусловлено тем, что глиобластома характеризуется высокой резистентностью к химиотерапии. Одной из ключевых причин этого является устойчивость к окислительному стрессу.

Цель. Систематизация данных о редокс-зависимых механизмах развития химиорезистентности глиобластомы, а также анализ перспективы применения препаратов, дестабилизирующих редокс-гомеостаз, в терапии глиобластомы.

Обсуждение. Клетки опухоли в ответ на действие терапии активируют антиоксидантные системы, тем самым сдерживая выброс активных форм кислорода, вызванного действием химиопрепаратов, стабилизируя внутриклеточный окислительно-восстановительный гомеостаз (редокс-гомеостаз) и предотвращая развитие окислительного стресса. В связи с этим применение соединений, обеспечивающих усиление генерации внутриклеточных активных форм кислорода либо подавляющих активность ключевых компонентов антиоксидантной защиты, представляется перспективным подходом для сенсibilизации опухоли к терапии. Ряд препаратов на основе таких соединений в качестве монотерапии либо в комбинации с другими подходами показывает свою эффективность в доклинических испытаниях и демонстрирует эффективность для терапии пациентов с глиобластомой в ходе клинических исследований.

Выводы. Поиск более селективных ингибиторов антиоксидантных систем, оптимизация их доставки в опухоль и стратификация пациентов на основе молекулярно-генетических и биохимических маркеров редокс-гомеостаза опухоли может увеличить эффективность терапии глиобластомы.

Ключевые слова: глиобластома; химиотерапия; окислительный стресс; антиоксидантные системы; устойчивость к терапии

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INTRODUCTION

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. According to current data, the median survival of patients from the date of diagnosis ranges 12–14 months, with the proportion of GBM patients living longer than five years being 6.8%. The latter figure strongly depends on the age at which the diagnosis was made [1]. The standard treatment for GBM involves three stages: surgical tumor resection followed by radiation and chemotherapy. Currently, temozolomide (TMZ) is the primary chemotherapeutic agent for first-line treatment of GBM, the use of which has increased the median patient survival by only two months, from 12.1 to 14.6 months. Thus, the current therapy does not show high effectiveness, mainly due to high inter- and intra-tumoral heterogeneity, the invasive nature of the tumor, its molecular-genetic plasticity, as well as its pronounced resistance to radiation and chemotherapy [2, 3].

The cytotoxic mechanism of TMZ action is based on the process of DNA alkylation. Thanks to its lipophilic nature, TMZ can cross the blood–brain barrier and is hydrolyzed with the release of an active metabolite that methylates guanine and adenine in DNA. Particularly significant is the O⁶-methylation of guanine at the sixth position (O⁶), forming O⁶-methylguanine, which pairs with thymine instead of cytosine. The mismatch repair system in this case replaces O⁶-methylguanine with adenine, thereby introducing mutations. Furthermore, excessive activity of repair systems leads to single- and double-strand breaks, significantly inhibiting gene expression and replication. This destabilizes the genetic material and disrupts the cell cycle, triggering apoptosis and tumor cell death [4]. However, almost invariably, after a short period of time, the tumor resumes its growth and acquires a more aggressive phenotype due to the presence of intrinsic or acquired mechanisms of TMZ resistance. As a result, patients with GBM inevitably experience tumor recurrence [5].

One of the most well-studied mechanisms of TMZ resistance is mediated by the O⁶-methylguanine-DNA methyltransferase (MGMT), an enzyme that removes the methyl group from O⁶-methylguanine. This enzyme neutralizes DNA damage caused by alkylating agents and promotes the development of tumor cell resistance

to these agents [5]. However, MGMT expression is suppressed when the promoter of its gene is methylated. The use of TMZ for treating GBM patients with a methylated MGMT promoter has increased their median survival to 21.2 months, compared to 14 months for patients with an unmethylated promoter. Thus, the methylation status of the MGMT promoter is one of the recognized and clinically applicable prognostic markers for GBM treatment [6]. However, although some patients showed initial improvement after TMZ treatment, tumor growth was observed to resume, leading to recurrence due to the development of acquired resistance [7].

The repair activity of MGMT is an important, but not the sole, factor that contributes to the development of TMZ resistance. In recent years, many other molecular mechanisms of GBM chemoresistance to TMZ have been studied [8]. One such mechanism is the adaptation of redox metabolism in cancer cells in response to TMZ treatment [9]. Cellular models of GBM have shown that TMZ-induced damage of genetic material triggers an increased production of reactive oxygen species (ROS) and leads to the development of oxidative stress, DNA damage, lipid membrane damage, and protein damage, as well as the activation of pro-apoptotic cascades [10]. Tumor cells, in turn, can enhance the activity of antioxidant systems, reducing the level of oxidative stress [11]. In this regard, targeting redox metabolism with the purpose of shifting the balance toward oxidative stress and cancer cell death may become an effective approach for anticancer therapy.

In this study, we carry out a review of the redox-dependent mechanisms of chemoresistance development in GBM, as well as analyze the potential of drugs that destabilize redox homeostasis for glioblastoma therapy.

MATERIALS AND METHODS

The search for scientific literature was conducted in scientific databases (PubMed, Google Scholar) using the following keywords: glioblastoma, chemotherapy resistance, oxidative stress, antioxidant system, redox metabolism. As a result, 60 publications were selected and analyzed, including review and original research articles, as well as clinical studies published between 2004–2025 in international peer-reviewed journals.

RESULTS AND DISCUSSION

Key components of cellular redox balance

ROS production is an integral part of cellular metabolism. ROS are formed as a result of the partial reduction of molecular oxygen O_2 . Two main types of ROS with the greatest physiological significance are the superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), from which all other forms originate. The superoxide anion radical $O_2^{\cdot-}$ is generated in cells due to the leakage of electrons from complexes I and III in the mitochondria, as well as through the directed and controlled production of $O_2^{\cdot-}$ by the members of NADPH oxidase (NOX) family [12]. The superoxide anion radical $O_2^{\cdot-}$ itself exhibits a relatively low reactivity; however, its interaction with other reactive species, such as H_2O_2 or nitric oxide (II) (NO), can form highly reactive compounds, including hydroxyl radical $OH^{\cdot}$ or peroxynitrite $ONOO^-$. These compounds can destabilize the structures of proteins, DNA, and lipids [12]. The superoxide anion radical, $O_2^{\cdot-}$, dismutates either spontaneously or with the help of superoxide dismutase (SOD) enzymes into O_2 and H_2O_2 , and this pathway serves as the primary source of intracellular H_2O_2 . The relative stability of H_2O_2 compared to other ROS and its ability to diffuse over long distances make this molecule a participant in various intracellular signaling and regulatory processes. At the same time, H_2O_2 predominantly interacts with SH-groups of cysteine residues in proteins, altering their conformation and functional activity [13]. The accepted physiological concentration of H_2O_2 in the cytoplasm ranges 1–10 nM. When the intracellular concentration of H_2O_2 increases to 50–70 nM, it activates the processes related to proliferation, survival, and migration, while H_2O_2 levels exceeding 100 nM for an extended period of time trigger cell death [13]. Excessive concentrations of H_2O_2 can interact with other ROS, forming highly reactive compounds, and irreversibly oxidize proteins, leading to oxidative stress and cell death. In response, cells have developed diverse mechanisms for multi-level regulation of intracellular ROS content [14].

Important enzymes that metabolize ROS are superoxide dismutase (SOD), which catalyzes the dismutation of $O_2^{\cdot-}$ into O_2 and H_2O_2 , and catalase, which converts excess H_2O_2 into O_2 and H_2O . These enzymes neutralize high levels of ROS rather rapidly and are effective in combating significant oxidative stress [15]. For more precise regulation of H_2O_2 levels, enzymatic systems which are sensitive to low concentrations of H_2O_2 have developed additional levels of activity regulation. These include the glutathione-dependent and thioredoxin-dependent antioxidant systems [16].

The key enzymes of the glutathione-dependent antioxidant defense system are glutathione peroxidases (GPX). They reduce H_2O_2 to H_2O , using glutathione (GSH) as an electron donor. The oxidation of GSH leads to the condensation of SH-groups from cysteine residues of

two GSH molecules, forming an oxidized form of glutathione (GSSG). The reduction of GSSG is performed by the FAD-containing enzyme glutathione reductase using NADPH [17].

Glutathione is a tripeptide consisting of glycine, cysteine, and glutamate. The synthesis of this antioxidant is highly dependent on the availability of cysteine, which is mediated by the cystine/glutamate antiporter SLC7A11 (xCT). The intracellular glutathione content ranges 1–15 mM, which helps maintain the SH-groups of most intracellular proteins in their reduced state [18]. In addition to reducing the active sites of GPX, glutathione conjugates with cysteine residues of various proteins through the action of glutathione S-transferase enzymes, while the removal of glutathione and the reduction of protein cysteines are carried out by the low-molecular-weight oxidoreductases referred to as glutaredoxins. These processes, on the one hand, protect protein SH-groups from oxidative stress, and on the other hand, provide redox-dependent regulation of many protein kinases, transcription factors, signaling proteins, and metabolic enzymes [19].

The thioredoxin-based antioxidant defense system relies on the functioning of peroxiredoxins (PRDX). Under normal conditions, they can neutralize up to 90% of ROS, reducing H_2O_2 to H_2O through the oxidation of cysteine residues in the active sites. Their reduction requires a special type of low-molecular-weight reductases — thioredoxins. In turn, thioredoxin reduction is performed by thioredoxin reductases (TrxR), which accept electrons from NADPH [20].

The functioning of glutathione and thioredoxin antioxidant systems depends on an adequate level of NADPH, which is primarily maintained by the activity of pentose phosphate pathway enzymes, such as glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [21]. Furthermore, in addition to the main antioxidant systems, there are proteins that, rather than directly inactivating ROS, either limit their production or eliminate metabolites formed with ROS involvement in the cell. These proteins include enzymes that metabolize xenobiotics, such as aldehyde dehydrogenases (ALDH), aldo-keto reductases (AKR), carbonyl reductases (CBR), and NADPH:quinone reductases (NQO), which mitigate the consequences of ROS cytotoxicity by preventing the accumulation of lipid oxidation products, disrupting the electron transport chain, and depleting the glutathione pool [22].

Cellular systems for maintaining stable ROS levels are strictly controlled by a complex network of transcription factors. One of the primary regulators of cellular redox homeostasis is the nuclear factor NRF2. Its activity is strictly regulated by the KEAP1 protein, which targets NRF2 for proteasomal degradation in the absence of oxidative stress. NRF2 activation occurs in response to increased ROS levels, which destabilize the KEAP1 structure, allowing the released NRF2 to translocate to the nucleus. In the nucleus, NRF2 interacts with sequences

in the promoter regions of its target genes, known as antioxidant response elements (ARE), and activates genes that encode ROS scavengers, such as catalase, glutathione peroxidases, and peroxiredoxins, as well as proteins involved in glutathione metabolism and the pentose phosphate pathway. This enables a multifaceted response to elevated ROS levels in cells [23].

Among other significant regulators of cellular redox processes are various transcription factors and master regulators activated in response to stress stimuli, such as hypoxia (hypoxia-inducible factor, HIF-1 α), endoplasmic reticulum stress and unfolded protein accumulation (heat shock protein, HSP1), inflammation, ATP deficiency, and genetic material damage (nuclear factor NF- κ B). To counteract oxidative stress, they shift the metabolism from oxidative phosphorylation to glycolysis, reducing ROS production by mitochondrial electron transport chain complexes, enhance glutathione synthesis and reduction, and activate antioxidant defense systems in mitochondria and the endoplasmic reticulum. At the same time, other regulators, such as p53, Jun, Fos, ATF, and FOXO proteins, which are responsible for regulating the cell cycle, proliferation, differentiation, and apoptosis, are also capable of activating the expression of antioxidant systems in response to induced oxidative stress [22]. Although all these factors respond to different threshold levels of ROS and are unlikely to be activated simultaneously during oxidative stress, they coordinate their activities in response to various types of stress.

Thus, cells regulate the maintenance of moderate ROS levels through multiple mechanisms. It is known that the activity of many proteins involved in the antioxidant systems, as well as transcription factors ensuring their high expression, are enhanced in tumors, including GBM, indicating their role in tumor growth, invasiveness, and therapy resistance [22].

Mechanisms of redox-mediated chemoresistance in glioblastoma cells

In a response to the cytotoxic effects of chemotherapy, GBM utilizes several mechanisms involving antioxidant systems and mitochondria [24].

Chemotherapy-treated GBM cells show an increased expression of NRF2. The overexpression of NRF2 is observed in recurrent tumors, being correlated with GBM therapy resistance and low patient survival [25]. Genome-wide CRISPR/Cas9 screening of genes conferring GBM chemoresistance has also confirmed NRF2 to be one of the key factors ensuring tumor survival when exposed to TMZ [26].

As mentioned previously, the antioxidant function of NRF2 is associated with the activation of the glutathione and thioredoxin antioxidant systems, as well as the system of enzymes maintaining sufficient NADPH concentration. These three systems contribute to the development and maintenance of a resistant phenotype

in GBM. For example, the disruption of glutathione synthesis by suppressing NRF2 expression or the inhibition of γ -glutamylcysteine ligase can sensitize cells to TMZ [11]. It was also shown that an increase in the expression of the cystine/glutamate antiporter xCT, which imports cysteine for glutathione synthesis, correlates with the expression of markers of a more aggressive GBM phenotype and reprograms tumor cells into a chemotherapy-resistant subtype [27]. Enzymes that help maintain cellular redox homeostasis and use glutathione as a cofactor also contribute to the development of a chemoresistant phenotype in GBM. Thus, the high activity of glutathione reductase, which replenishes the reduced glutathione pool, corresponded to a low tumor sensitivity to TMZ [28].

Furthermore, high expression of thioredoxin reductase and thioredoxin correlates with low survival rates in patients with various brain tumors, including GBM [29]. Inhibition of thioredoxin sensitizes the tumor to the action of chemotherapeutic agents [30].

It was been observed that GBM can overcome TMZ-induced oxidative stress by utilizing a mechanism of mitochondrial metabolic switching. It is known that TMZ can methylate not only nuclear but also mitochondrial DNA. In response to this stress, the GBM mitochondria remodel the electron transport chain (ETC). For instance, in TMZ-resistant cells, a reduced activity of complex I and ATP synthase, coupled with an enhanced activity of complexes III and IV, was observed. This ensures a more efficient electron flow through the ETC for ATP energy production and reduced ROS generation [31]. In addition, increased expression of the mitochondrial isoform of superoxide dismutase, SOD2, was demonstrated in TMZ-resistant GBM cells. This expression was not coordinated by NRF2, but rather by Sp1, another transcription factor, which mediates stress response. This finding reveals a new mechanism for the emergence of redox-associated resistance in GBM [32].

Finally, enzymes of detoxification systems, such as aldehyde dehydrogenases (ALDH), play a significant role in the development of GBM chemoresistance. Enzymes of this family catalyze the oxidation of intracellular aldehydes to their corresponding carboxylic acids, neutralizing lipid peroxidation products, such as malondialdehyde and 4-hydroxy-2-nonenal, and thereby preventing their interaction with enzyme active sites and destabilization of protein homeostasis [33]. At the same time, increased expression of one of the ALDH isoforms, ALDH1A3, is an important factor in maintaining the stem-like properties of tumor cells, serving as a marker of a more aggressive mesenchymal phenotype in GBM [34].

The presented data indicate that increased expression of antioxidant systems in GBM is a significant factor contributing to GBM resistance to TMZ chemotherapy. Therefore, the identification of compounds that destabilize antioxidant activity in GBM, for their use in combination with established chemotherapeutic agents as

a therapy, is a promising solution for overcoming tumor resistance.

Existing approaches to glioblastoma therapy based on drugs inducing oxidative stress

Currently, there exist numerous synthetic and natural compounds with a pro-oxidant effect induced by the production of ROS or inhibition of tumor cell antioxidant systems. Drugs based on some of these compounds are undergoing preclinical and clinical trials for GBM therapy.

Since the intracellular redox homeostasis of GBM largely depends on the quantity and status of glutathione within the cell, destabilization of the synthesis of this tripeptide and disruption of the delivery of its precursor molecules appear to be an effective way to block the glutathione-dependent antioxidant system. Reducing glutathione content in cells induces ferroptosis, a type of non-apoptotic type of cell death, associated with lipid peroxidation, which is often observed in malignant tumor cells [35]. One of the components that directly suppresses the maintenance of the glutathione pool is L-buthionine sulfoximine (BSO), which irreversibly inhibits γ -glutamyl cysteine synthetase, an enzyme that catalyzes the rate-limiting step of glutathione synthesis. Although BSO has not demonstrated significant efficacy in monotherapy, its use has enhanced the effect of cisplatin and carboplatin in experiments on GBM cell cultures, suggesting that BSO may be a potential candidate for use in combination with primary GBM therapy [36].

Another promising strategy for suppressing the glutathione system in GBM involves reducing the availability of cysteine for glutathione synthesis, e.g., by inhibiting the activity of the xCT cystine/glutamate antiporter. Sulfasalazine was used to inhibit xCT, effectively targeting this transporter in GBM cells. However, clinical trials of this drug have currently been discontinued due to its high systemic toxicity [37]. Another xCT inhibitor that has been tested in GBM models is erastin, which, in addition to suppressing cysteine import into cells, destabilizes mitochondrial voltage-dependent anion channels (VDAC) and disrupts the integrity of the mitochondrial outer membrane [38]. Erastin also inhibits cystathionine- γ -lyase, a key enzyme in sulfur-containing amino acid metabolism that catalyzes the conversion of cystathionine into cysteine, thus completely blocking the possibility of obtaining cysteine for glutathione synthesis [39]. Erastin has demonstrated a more potent cytotoxic effect than sulfasalazine, while also sensitizing GBM to TMZ [39]. Finally, RSL-3, another ferroptosis inducer that targets GPX4 inhibition, is currently being tested as a potential GBM therapy. This compound effectively suppressed GBM growth *in vitro* and enhanced the efficacy of TMZ [40].

The thioredoxin antioxidant system has been recognized as a promising target for combating malignant

tumors. The activity of peroxiredoxins, the most abundant antioxidant proteins in cells, is almost entirely dependent on the amount of reduced thioredoxin, the availability of which is regulated by TrxR. Therefore, impairing the activity of such a crucial enzyme can lead to an almost complete destabilization of cellular redox homeostasis. The selenocysteine-containing active site of TrxR is susceptible to irreversible inhibition by alkylating agents, certain natural compounds, such as curcumin and piperlongumine, as well as metal-organic complexes based on mercury, gold, silver, platinum, and gadolinium [41]. One such gold complex is auranofin, an anti-inflammatory drug. The pronounced pro-oxidant and antitumor effect of auranofin was repeatedly demonstrated on GBM cell cultures, including those with a therapy-resistant phenotype [42, 43].

Drugs based on compounds that induce enhanced generation and accumulation of ROS have shown antitumor effects in GBM in preclinical and clinical studies. One such drug is arsenic trioxide (As_2O_3), which triggers ROS release and suppresses the activity of various antioxidant enzymes. During phase I and II clinical trials of the efficacy of a combination of As_2O_3 with radiation therapy and TMZ, no significant side effects were identified. An increase in the overall and relapse-free survival was demonstrated in GBM patients [44].

High-dose ascorbate is a promising pro-oxidant agent for tumor therapy. Its use induces enhanced generation of H_2O_2 in tumor cells, leading to their death. Ascorbate also increases tumor sensitivity to radiation and chemotherapy. The combination of ascorbate with TMZ was shown to be effective in clinical trials for patients with primary GBM [45]. Phytocompounds, such as resveratrol [46] and cannabidiol [27], also sensitize tumors to TMZ through ROS production, as demonstrated in various *in vitro* and *in vivo* GBM models.

Another promising target for destabilizing redox homeostasis in GBM is NADPH:quinone oxidoreductase 1 (NQO1). This is an enzyme of the xenobiotic detoxification and antioxidant defense system, often overexpressed in tumors [47]. Certain natural compounds from the quinone class, such as β -lapachone or tanshindiol [48], are substrates of NQO1. Their metabolism leads to the depletion of the NADPH pool, which is necessary for the function of antioxidant systems. This results in oxidative stress, which sensitizes tumors to TMZ [49]. In cell cultures and GBM xenograft models, the use of quinone derivatives, such as menadione [50] or coenzyme Q [51], in combination with ascorbate, was shown to selectively affect tumor cells and suppress their growth and proliferation through the induction of oxidative stress.

Thus, preclinical and clinical studies of drugs based on compounds that destabilize the redox homeostasis of cancer cells have shown that their use in combination with conventional radiation therapy and TMZ chemotherapy can reduce tumor resistance to chemotherapy, hamper the transformation of the tumor into a more

aggressive phenotype, and improve survival rates in patients with GBM.

Future research directions for glioblastoma therapy using redox-targeting drugs

The adaptation of redox metabolism to stress conditions induced by chemotherapy, mediated by the activation of antioxidant systems, is a key mechanism of GBM resistance. Therefore, the development of therapeutic strategies aimed at inhibiting these systems for effectively inducing oxidative stress is a highly relevant task. In this field, three main directions can be distinguished: development of selective inhibitors of antioxidant system components, optimization of local delivery of these inhibitors, and selection of personalized approaches to GBM therapy based on the data on the expression of antioxidant systems and the assessment of redox status in tumors.

Currently, approaches aimed at selective inhibition of such components of the antioxidant system as thioredoxin reductase are being actively developed. Understanding the molecular structure of the TrxR active site and its interaction mechanisms with inhibitors opens possibilities for designing and discovering new drugs. As was discussed above, auranofin, a gold-containing drug, irreversibly interacts with the selenocysteine residue in the TrxR active site. Along with that, organic mercury compounds, such as thimerosal (TmHg) and its metabolite ethylmercury (EtHg), are also considered as inhibitors. These substances exhibit a high affinity for selenocysteine residues in the active site of TrxR, while also having the ability to cross the blood–brain barrier. These properties could enable effective GBM therapy. Studies into GBM cell cultures showed that the exposure to micromolar concentrations of TmHg and EtHg leads to a significant increase in oxidative stress and cell death via apoptosis. Furthermore, exhibiting no significant toxicity, TmHg is widely used as a preservative in many drug components, making its use as an antitumor agent feasible [52]. Future research should focus on both investigating the drugs mentioned above and on searching for new selective TrxR inhibitors.

Many inhibitors of antioxidant systems have a rather limited effect due to poor penetration through the blood–brain barrier (BBB) and the inability to achieve effective concentrations in brain tissues. Strategies for improving drug delivery across the BBB include enhancing the properties of the substance itself and its formulation, e.g., increasing its solubility and stability in liquid media by adding polysorbates or ethanol. Direct chemical modification of the substance by adding lipophilic groups can also be used. One promising direction consists in encapsulating the active substance to form nanoparticles [53]. For this purpose, polymer and PLGA particles, as well as such inorganic nanoparticles as magnetic or gold silicon nanoparticles are employed. However, the

most popular method for delivering active agents to the brain is the use of cationic liposomes. Another promising strategy is the application of active targeting implying the attachment of ligands to nanoparticles that specifically bind to the BBB receptors, such as transferrin or insulin [41]. PLGA nanoparticles with transferrin loaded with drugs like doxorubicin and paclitaxel significantly suppressed glioma growth in *in vivo* experiments [54]. Preclinical studies demonstrated the efficacy of methods that temporarily and reversibly increase BBB permeability using focused ultrasound (FUS) or osmotically active components, such as mannitol [41]. Almost all the aforementioned strategies can be applied to deliver the inhibitors of antioxidant systems; however, they require adaptation of the methods to specific molecules.

Development of individualized approaches to GBM therapy requires the assessment of parameters that reflect the redox homeostasis status of tumor cells [55]. As mentioned previously, increased NRF2 expression correlates with GBM therapy resistance and low patient survival [9]. At the same time, high NRF2 expression in GBM is associated with increased sensitivity of tumor cells to ferroptosis. Therefore, the use of ferroptosis-inducing drugs may sensitize tumors with high NRF2 expression to therapy [56]. In order to determine NRF2 activity in tumor cells and, accordingly, predict the effectiveness of therapy for a specific patient, the expression of target genes controlled by this transcription factor can be evaluated, such as the catalytic (GCLC) and modifier (GCLM) subunits of γ -glutamyl cysteine ligase, which catalyze the rate-limiting step of glutathione synthesis; NADPH:quinone oxidoreductase 1 (NQO1); heme oxygenase 1 (HMOX1); thioredoxin reductase 1 (TXNRD1); and sulfiredoxin 1 (SRXN1). Overexpression of these genes suggests hyperactivation of the NRF2 pathway and may indicate the “redox sensitivity” of the tumor [57].

When predicting the response to chemotherapy and, therefore, developing personalized approaches to treating various oncological diseases with redox agents, the status of the glutathione system in tumor cells can serve as a predictive marker [58]. Assessment of the expression of genes related to glutathione metabolism [59], as well as direct measurement of the total glutathione content and the ratio of its oxidized and reduced forms in tumor tissue, can provide valuable information about the antioxidant reserves of the tumor. A low basal level of intracellular glutathione may indicate a potential vulnerability to glutathione synthesis inhibitors [43]. The activity of glutathione peroxidases, glutathione reductase, and glutathione S-transferases provides additional information about functioning of this antioxidant system [60].

Combination therapy including both TrxR inhibitors and GSH synthesis inhibitors may be more effective than standard therapy for patients with high NRF2 expression and its target genes, as well as a low GSH/GSSG ratio.

CONCLUSION

The development of an effective therapy for glioblastoma, one of the most aggressive tumors, remains a significant challenge. Conventional therapy for such tumors based on the use of temozolomide only slightly improves clinical outcomes in patients. The high activity of the antioxidant systems in tumor cells restrains oxidative stress induced by standard chemotherapy agents, ensuring the survival and chemoresistance of the tumor. The conducted review of available data on redox-dependent mechanisms of chemoresistance development in GBM, as well as the analysis of prospects for drugs that destabilize tumor re-

dox homeostasis in GBM therapy, has been shown that the use of drugs aimed at destabilizing the redox homeostasis of GBM cells, either as monotherapy or in combination with conventional chemotherapeutic agents, can improve survival rates in GBM patients. Nevertheless, the development of more effective therapeutic regimens using inhibitors of antioxidant systems or pro-oxidants is still necessary. The search for more selective and stable inhibitors of key antioxidant enzymes, the optimization of approaches for their delivery to the tumor, as well as the stratification of GBM patients based on biomarkers associated with redox homeostasis, open prospects for more effective GBM therapy.

References

- Wen PY, Weller M, Lee EQ, Alexander BM, Barnholtz-Sloan JS, Barthel FP, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. *Neuro-Oncology*. 2020;22(8):1073–113. <https://doi.org/10.1093/neuonc/noaa106>
- Dymova MA, Kuligina EV, Richter VA. Molecular Mechanisms of Drug Resistance in Glioblastoma. *International Journal of Molecular Sciences*. 2021;22(12):6385. <https://doi.org/10.3390/ijms22126385>
- Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *The Lancet Oncology*. 2009;10(5):459–66. [https://doi.org/10.1016/S1470-2045\(09\)70025-7](https://doi.org/10.1016/S1470-2045(09)70025-7)
- Zhang J, Stevens MFG, Bradshaw TD. Temozolomide: Mechanisms of Action, Repair and Resistance. *Current Molecular Pharmacology*. 2012;5(1):102–14. <https://doi.org/10.2174/1874467211205010102>
- Lee SY. Temozolomide resistance in glioblastoma multi-forme. *Genes & Diseases*. 2016;3(3):198–210. <https://doi.org/10.1016/j.gendis.2016.04.007>
- Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. *Journal of Clinical Oncology*. 2013;31(32):4085–91. <https://doi.org/10.1200/JCO.2013.49.6968>
- Chien CH, Hsueh WT, Chuang JY, Chang KY. Dissecting the mechanism of temozolomide resistance and its association with the regulatory roles of intracellular reactive oxygen species in glioblastoma. *Journal of Biomedical Science*. 2021;28(1):18. <https://doi.org/10.1186/s12929-021-00717-7>
- Singh N, Miner A, Hennis L, Mittal S. Mechanisms of temozolomide resistance in glioblastoma — a comprehensive review. *Cancer Drug Resistance*. 2021;4(1):17–43. <https://doi.org/10.20517/cdr.2020.79>
- Almeida Lima K, Osawa IYA, Ramalho MCC, de Souza I, Guedes CB, de Souza Filho CHD, et al. Temozolomide Resistance in Glioblastoma by NRF2: Protecting the Evil. *Biomedicine*. 2023;11(4):1081. <https://doi.org/10.3390/biomedicine11041081>
- Zhang W, Wang Z, Shu F, Jin Y, Liu H, Wang Q, et al. Activation of AMP-activated Protein Kinase by Temozolomide Contributes to Apoptosis in Glioblastoma Cells via p53 Activation and mTORC1 Inhibition*. *Journal of Biological Chemistry*. 2010;285(52):40461–71. <https://doi.org/10.1074/jbc.M110.164046>
- Rocha CRR, Kajitani GS, Quinet A, Fortunato RS, Menck CFM. NRF2 and glutathione are key resistance mediators to temozolomide in glioma and melanoma cells. *Oncotarget*. 2016;7(30):48081–92. <https://doi.org/10.18632/oncotarget.10129>
- Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews. Molecular Cell Biology*. 2020;21(7):363–83. <https://doi.org/10.1038/s41580-020-0230-3>
- Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biology*. 2017;11:613–9. <https://doi.org/10.1016/j.redox.2016.12.035>
- Chandimali N, Bak SG, Park EH, Lim HJ, Won YS, Kim EK, et al. Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discovery*. 2025;11(1):19. <https://doi.org/10.1038/s41420-024-02278-8>
- Baker A, Lin CC, Lett C, Karpinska B, Wright MH, Foyer CH. Catalase: A critical node in the regulation of cell fate. *Free Radical Biology and Medicine*. 2023;199:56–66. <https://doi.org/10.1016/j.freeradbiomed.2023.02.009>
- Ren X, Zou L, Zhang X, Branco V, Wang J, Carvalho C, et al. Redox signaling mediated by thioredoxin and glutathione systems in the central nervous system. *Antioxidants & Redox Signaling*. 2017;27(13):989–1010. <https://doi.org/10.1089/ars.2016.6925>
- Kennedy L, Sandhu JK, Harper ME, Cuperlovic-Culf M. Role of Glutathione in Cancer: From Mechanisms to Therapies. *Biomolecules*. 2020;10(10):1429. <https://doi.org/10.3390/biom10101429>
- Østergaard H, Tachibana C, Winther JR. Monitoring disulfide bond formation in the eukaryotic cytosol. *Journal of Cell Biology*. 2004;166(3):337–45. <https://doi.org/10.1083/jcb.200402120>
- Ogata FT, Branco V, Vale FF, Coppo L. Glutaredoxin: Discovery, redox defense and much more. *Redox Biology*. 2021;43:101975. <https://doi.org/10.1016/j.redox.2021.101975>
- Perkins A, Nelson KJ, Parsonage D, Poole LB, Karplus PA. Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. *Trends in Biochemical Sciences*. 2015;40(8):435–45. <https://doi.org/10.1016/j.tibs.2015.05.001>
- Purohit V, Simeone DM, Lyssiotis CA. Metabolic Regulation of Redox Balance in Cancer. *Cancers*. 2019;11(7):955. <https://doi.org/10.3390/cancers11070955>

22. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative Stress in Cancer. *Cancer Cell*. 2020;38(2):167–97. <https://doi.org/10.1016/j.ccell.2020.06.001>
23. Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 System: a Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis. *Physiological Reviews*. 2018;98(3):1169–203. <https://doi.org/10.1152/physrev.00023.2017>
24. Campos-Sandoval JA, Gómez-García MC, de los Santos-Jiménez J, Matés JM, Alonso FJ, Márquez J. Antioxidant responses related to temozolomide resistance in glioblastoma. *Neurochemistry International*. 2021;149:105136. <https://doi.org/10.1016/j.neuint.2021.105136>
25. Cong ZX, Wang HD, Zhou Y, Wang JW, Pan H, Zhang DD, et al. Temozolomide and irradiation combined treatment-induced Nrf2 activation increases chemoradiation sensitivity in human glioblastoma cells. *Journal of Neuro-Oncology*. 2014;116(1):41–8. <https://doi.org/10.1007/s11060-013-1260-x>
26. Rocha CRR, Rocha AR, Silva MM, Gomes LR, Latancia MT, Andrade-Tomaz M, et al. Revealing Temozolomide Resistance Mechanisms via Genome-Wide CRISPR Libraries. *Cells*. 2020;9(12):2573. <https://doi.org/10.3390/cells9122573>
27. Singer E, Judkins J, Salomonis N, Matlaf L, Soteropoulos P, McAllister S, et al. Reactive oxygen species-mediated therapeutic response and resistance in glioblastoma. *Cell Death & Disease*. 2015;6(1):e1601. <https://doi.org/10.1038/cddis.2014.566>
28. Zhu Z, Du S, Du Y, Ren J, Ying G, Yan Z. Glutathione reductase mediates drug resistance in glioblastoma cells by regulating redox homeostasis. *Journal of Neurochemistry*. 2018;144(1):93–104. <https://doi.org/10.1111/jnc.14250>
29. Yao A, Storr SJ, Al-hadyan K, Rahman R, Smith S, Grundy R, et al. Thioredoxin System Protein Expression Is Associated with Poor Clinical Outcome in Adult and Paediatric Gliomas and Medulloblastomas. *Molecular Neurobiology*. 2020;57(7):2889–901. <https://doi.org/10.1007/s12035-020-01928-z>
30. Haas B, Schütte L, Wos-Maganga M, Weickhardt S, Timmer M, Eckstein N. Thioredoxin Confers Intrinsic Resistance to Cytostatic Drugs in Human Glioma Cells. *International Journal of Molecular Sciences*. 2018;19(10):2874. <https://doi.org/10.3390/ijms19102874>
31. Oliva CR, Moellering DR, Gillespie GY, Griguer CE. Acquisition of Chemoresistance in Gliomas Is Associated with Increased Mitochondrial Coupling and Decreased ROS Production. *PLOS One*. 2011;6(9):e24665. <https://doi.org/10.1371/journal.pone.0024665>
32. Chang KY, Hsu TI, Hsu CC, Tsai SY, Liu JJ, Chou SW, et al. Specificity protein 1-modulated superoxide dismutase 2 enhances temozolomide resistance in glioblastoma, which is independent of O⁶-methylguanine-DNA methyltransferase. *Redox Biology*. 2017;13:655–64. <https://doi.org/10.1016/j.redox.2017.08.005>
33. Rasper M, Schäfer A, Piontek G, Teufel J, Brockhoff G, Ringel F, et al. Aldehyde dehydrogenase 1 positive glioblastoma cells show brain tumor stem cell capacity. *Neuro-Oncology*. 2010;12(10):1024–33. <https://doi.org/10.1093/neuonc/noq070>
34. Mao P, Joshi K, Li J, Kim SH, Li P, Santana-Santos L, et al. Mesenchymal glioma stem cells are maintained by activated glycolytic metabolism involving aldehyde dehydrogenase 1A3. *Proceedings of the National Academy of Sciences*. 2013;110(21):8644–9. <https://doi.org/10.1073/pnas.1221478110>
35. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell*. 2012;149(5):1060–72. <https://doi.org/10.1016/j.cell.2012.03.042>
36. Rocha CRR, Garcia CCM, Vieira DB, Quinet A, de Andrade-Lima LC, Munford V, et al. Glutathione depletion sensitizes cisplatin- and temozolomide-resistant glioma cells *in vitro* and *in vivo*. *Cell Death & Disease*. 2014;5(10):e1505. <https://doi.org/10.1038/cddis.2014.465>
37. Robe PA, Martin DH, Nguyen-Khac MT, Artesi M, Deprez M, Albert A, et al. Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of Sulfasalazine for the treatment of progressing malignant gliomas in adults. *BMC Cancer*. 2009;9(1):372. <https://doi.org/10.1186/1471-2407-9-372>
38. Yagoda N, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, Fridman DJ, et al. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature*. 2007;447(7146):865–9. <https://doi.org/10.1038/nature05859>
39. Chen L, Li X, Liu L, Yu B, Xue Y, Liu Y. Erastin sensitizes glioblastoma cells to temozolomide by restraining xCT and cystathionine-γ-lyase function. *Oncology Reports*. 2015;33(3):1465–74. <https://doi.org/10.3892/or.2015.3712>
40. Yang FC, Wang C, Zhu J, Gai QJ, Mao M, He J, et al. Inhibitory effects of temozolomide on glioma cells is sensitized by RSL3-induced ferroptosis but negatively correlated with expression of ferritin heavy chain 1 and ferritin light chain. *Laboratory Investigation*. 2022;102(7):741–52. <https://doi.org/10.1038/s41374-022-00779-7>
41. Chmelyuk N, Kordyukova M, Sorokina M, Sinyavskiy S, Meshcheryakova V, Belousov V, et al. Inhibition of Thioredoxin-Reductase by Auranofin as a Pro-Oxidant Anticancer Strategy for Glioblastoma: *In Vitro* and *In Vivo* Studies. *International Journal of Molecular Sciences*. 2025;26(5):2084. <https://doi.org/10.3390/ijms26052084>
42. Jamali F, Lan K, Daniel P, Petrecca K, Sabri S, Abdulkarim B. Synergistic Dual Targeting of Thioredoxin and Glutathione Systems Irrespective of p53 in Glioblastoma Stem Cells. *Antioxidants*. 2024;13(10):1201. <https://doi.org/10.3390/antiox13101201>
43. Martinez-Jaramillo E, Jamali F, Abdalbari FH, Abdulkarim B, Jean-Claude BJ, Telleria CM, et al. Pro-Oxidant Auranofin and Glutathione-Depleting Combination Unveils Synergistic Lethality in Glioblastoma Cells with Aberrant Epidermal Growth Factor Receptor Expression. *Cancers*. 2024;16(13):2319. <https://doi.org/10.3390/cancers16132319>
44. Kumthekar P, Grimm S, Chandler J, Mehta M, Marymont M, Levy R, et al. A phase II trial of arsenic trioxide and temozolomide in combination with radiation therapy for patients with malignant gliomas. *Journal of Neuro-Oncology*. 2017;133(3):589–94. <https://doi.org/10.1007/s11060-017-2469-x>
45. Allen BG, Bodeker KL, Smith MC, Monga V, Sandhu S, Hohl R, et al. First-in-Human Phase I Clinical Trial of Pharmacologic Ascorbate Combined with Radiation and Temozolomide for Newly Diagnosed Glioblastoma. *Clinical Cancer Research*. 2019;25(22):6590–7. <https://doi.org/10.1158/1078-0432.CCR-19-0594>
46. Yuan Y, Xue X, Guo RB, Sun XL, Hu G. Resveratrol enhances the antitumor effects of temozolomide in glioblastoma via ROS-dependent AMPK-TSC-mTOR signaling pathway. *CNS Neuroscience & Therapeutics*. 2012;18(7):536–46. <https://doi.org/10.1111/j.1755-5949.2012.00319.x>
47. Zhang K, Chen D, Ma K, Wu X, Hao H, Jiang S. NAD(P) H:Quinone Oxidoreductase 1 (NQO1) as a Therapeutic and

- Diagnostic Target in Cancer. *Journal of Medicinal Chemistry*. 2018;61(16):6983–7003.
<https://doi.org/10.1021/acs.jmedchem.8b00124>
48. Zhong B, Yu J, Hou Y, Ai N, Ge W, Lu JJ, et al. A novel strategy for glioblastoma treatment by induction of nptosis, an NQO1-dependent necrosis. *Free Radical Biology and Medicine*. 2021;166:104–15.
<https://doi.org/10.1016/j.freeradbiomed.2021.02.014>
 49. Dai Y, Min Y, Zhou L, Cheng L, Ni H, Yang Y, et al. Brain-targeting redox-sensitive micelles for codelivery of TMZ and β -lapachone for glioblastoma therapy. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2024;61:102772.
<https://doi.org/10.1016/j.nano.2024.102772>
 50. Ren X, Santhosh SM, Coppo L, Ogata FT, Lu J, Holmgren A. The combination of ascorbate and menadione causes cancer cell death by oxidative stress and replicative stress. *Free Radical Biology and Medicine*. 2019;134:350–8.
<https://doi.org/10.1016/j.freeradbiomed.2019.01.037>
 51. Sumiyoshi A, Shibata S, Lazarova D, Zhelev Z, Aoki I, Bakalova R. Tolerable treatment of glioblastoma with redox-cycling 'mitocans': a comparative study *in vivo*. *Redox Report: Communications in Free Radical Research*. 2023;28(1):2220531.
<https://doi.org/10.1080/13510002.2023.2220531>
 52. Pires V, Bramatti I, Aschner M, Branco V, Carvalho C. Thioredoxin Reductase Inhibitors as Potential Antitumors: Mercury Compounds Efficacy in Glioma Cells. *Frontiers in Molecular Biosciences*. 2022;9:889971.
<https://doi.org/10.3389/fmolb.2022.889971>
 53. Wei D, Zhang N, Qu S, Wang H, Li J. Advances in nanotechnology for the treatment of GBM. *Frontiers in Neuroscience*. 2023;17:1180943.
<https://doi.org/10.3389/fnins.2023.1180943>
 54. Cui Y, Xu Q, Chow PKH, Wang D, Wang CH. Transferrin-conjugated magnetic silica PLGA nanoparticles loaded with doxorubicin and paclitaxel for brain glioma treatment. *Biomaterials*. 2013;34(33):8511–20.
<https://doi.org/10.1016/j.biomaterials.2013.07.075>
 55. Chaiswing L, St Clair WH, St Clair DK. Redox Paradox: A Novel Approach to Therapeutics-Resistant Cancer. *Antioxidants & Redox Signaling*. 2018;29(13):1237–72.
<https://doi.org/10.1089/ars.2017.7485>
 56. de Souza I, Monteiro LKS, Guedes CB, Silva MM, Andrade-Tomaz M, Contieri B, et al. High levels of NRF2 sensitize temozolomide-resistant glioblastoma cells to ferroptosis via ABCC1/MRP1 upregulation. *Cell Death & Disease*. 2022;13(7):591.
<https://doi.org/10.1038/s41419-022-05044-9>
 57. Morgenstern C, Lastres-Becker I, Demirdöğen BC, Costa VM, Daiber A, Foresti R, et al. Biomarkers of NRF2 signalling: Current status and future challenges. *Redox Biology*. 2024;72:103134.
<https://doi.org/10.1016/j.redox.2024.103134>
 58. Kim Y, Ju H, Yoo SY, Jeong J, Heo J, Lee S, et al. Glutathione dynamics is a potential predictive and therapeutic trait for neoadjuvant chemotherapy response in bladder cancer. *Cell Reports Medicine*. 2023;4(10):101224.
<https://doi.org/10.1016/j.xcrm.2023.101224>
 59. Deng Z, Luo J, Ma J, Jin YN, Yu YV. Glutathione metabolism-related gene signature predicts prognosis and treatment response in low-grade glioma. *Aging*. 2024;16(11):9518–46.
<https://doi.org/10.18632/aging.205881>
 60. Chełchowska M, Gajewska J, Szczepanik E, Mazur J, Cychol A, Kuźniar-Pałka A, et al. Oxidative Stress Indicated by Nuclear Transcription Factor Nrf2 and Glutathione Status in the Blood of Young Children with Autism Spectrum Disorder: Pilot Study. *Antioxidants*. 2025;14(3):320.
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MACHINE LEARNING MODELS FOR STRATIFYING TRAUMATIC BRAIN INJURY SEVERITY BASED ON INTERLEUKIN-6 TEMPORAL DYNAMICS

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Introduction. Traumatic brain injury (TBI) remains a serious healthcare problem due to its heterogeneity and unpredictable outcomes. In TBI, secondary inflammatory cascades are frequently associated with the level of interleukin-6 (IL-6). However, its diagnostic and prognostic value at various time points after injury requires further investigation in athletes.

Objective. To evaluate the diagnostic accuracy and prognostic significance of IL-6 in stratifying TBI severity at various time points after injury with the use of machine learning models.

Materials and methods. A prospective cohort study was conducted among 89 male athletes, with a mean age of 28.7 ± 5.3 years. These athletes were actively engaged in contact sports (mixed martial arts and kudo) and had documented evidence of concussion or mild to moderate brain contusion. Sequential blood plasma collection was performed at 3, 6, 12, and 24 h post-injury. IL-6 concentration was determined using validated enzyme-linked immunosorbent assay (ELISA) protocols. In order to classify injury severity, models were trained using gradient boosting (XGBoost), logistic regression, and random forest algorithms. The accuracy of model responses was evaluated using ROC analysis. To identify the most significant time points, the SHAP (SHapley Additive exPlanations) feature importance method was applied. For patient prediction and stratification, a model based on a long short-term memory (LSTM) recurrent neural network was implemented. Statistical validation used the Kruskal–Wallis H -test ($H = 31.77$; $p < 0.001$) and Spearman's correlation analysis ($r_s = 0.81$; $p < 0.001$).

Results. The greatest discriminatory ability of IL-6 concentrations for moderate brain contusion was noted at 6 and 12 h. The XGBoost model achieved an area under the curve of 0.92 [95% CI: 0.88; 0.96], with a sensitivity of 87% and a specificity of 84%. The SHAP analysis revealed that IL-6 values at 6 and 12 h had the greatest impact on the model's predictions. Logistic regression and random forest yielded areas under the curve of 0.84 and 0.88, respectively. The identified diagnostic window between 6 and 12 h post-injury coincides with the peak of neuro-inflammatory activity.

Conclusions. The level of IL-6 measured within 6–12 h after TBI represents a specific biomarker for early stratification of injury severity. The integration of explainable machine learning approaches provides robust and clinically relevant decision support in neurotraumatology.

Keywords: IL-6; traumatic brain injury; machine learning; biomarker; long short-term memory; LSTM recurrent neural network; logistic regression; XGBoost

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МОДЕЛИ МАШИННОГО ОБУЧЕНИЯ ДЛЯ СТРАТИФИКАЦИИ ТЯЖЕСТИ ЧЕРЕПНО-МОЗГОВОЙ ТРАВМЫ НА ОСНОВЕ ВРЕМЕННОЙ ДИНАМИКИ ИНТЕРЛЕЙКИНА-6

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Введение. Черепно-мозговая травма (ЧМТ) остается серьезной проблемой в области здравоохранения в связи с ее гетерогенностью и непредсказуемостью исходов. Среди молекулярных маркеров интерлейкин-6 (ИЛ-6) часто ассоциируется с каскадами вторичных воспалительных реакций. Однако его диагностическая и прогностическая ценность в различные временные точки после травмы остается недостаточно изученной у спортсменов.

Цель. Оценка диагностической точности и прогностической значимости ИЛ-6 при стратификации тяжести ЧМТ в различные временные точки после травмы с использованием моделей машинного обучения.

Материалы и методы. Проведено проспективное когортное наблюдение 89 мужчин-спортсменов, активно занимающихся контактными видами спорта (смешанные единоборства и кудо), средний возраст $28,7 \pm 5,3$ года, с документированным подтверждением сотрясения мозга, ушибом мозга легкой и средней степени тяжести. Осуществляли последовательный забор плазмы крови через 3, 6, 12 и 24 ч после травмы. Концентрация ИЛ-6 определена с помощью валидированных протоколов для иммуноферментного

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анализа. Для решения задач классификации тяжести травмы были обучены модели на основе алгоритмов градиентного бустинга (XGBoost), логистической регрессии и случайного леса. Точность ответа моделей оценивалась с помощью ROC-анализа. Для определения наиболее значимых временных точек применялся метод оценки важности признаков SHAP (SHapley Additive exPlanations). Для прогнозирования и стратификации пациентов была реализована модель на основе рекуррентной нейронной сети с долгой краткосрочной памятью (LSTM). Статистическая валидация включала H -критерий Краскела – Уоллиса ($H = 31,77$; $p < 0,001$) и корреляционный анализ Спирмена ($r_s = 0,81$; $p < 0,001$).

Результаты. Концентрации ИЛ-6 через 6 и 12 ч продемонстрировали наибольшую дискриминационную способность при ушибе мозга средней степени тяжести. Модель XGBoost достигла AUC 0,92 [95% ДИ: 0,88; 0,96], чувствительности 87% и специфичности 84%. Анализ SHAP показал, что значения ИЛ-6 через 6 и 12 ч оказали наибольшее влияние на прогнозы модели. Логистическая регрессия и случайный лес дали AUC 0,84 и 0,88 соответственно. Выявленное диагностическое окно между 6 и 12 ч после травмы совпадает с пиком нейровоспалительной активности.

Выводы. Уровень ИЛ-6, измеренный в течение 6–12 ч после ЧМТ, представляет собой специфический биомаркер для ранней стратификации тяжести травмы. Интеграция объяснимых подходов машинного обучения обеспечивает надежную и клинически значимую поддержку принятия решений в нейротравматологии.

Ключевые слова: ИЛ-6; черепно-мозговая травма; машинное обучение; биомаркер; рекуррентная нейронная сеть с долгой краткосрочной памятью; логистическая регрессия; XGBoost

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INTRODUCTION

Traumatic brain injury (TBI) represents a global medical and socioeconomic problem that causes disability and premature mortality in millions of patients every year. According to the World Health Organization, up to 69 million people worldwide annually sustain a TBI of varying severity, with the majority of cases occurring in the working-age population (15–44 years). The diagnosis and stratification of TBI remain clinically challenging, especially in the absence of obvious neuroimaging changes and due to the variable clinical picture in the subacute and intermediate periods [1–3].

Biochemical markers are currently not used for diagnosis in Russian neurotraumatology. According to clinical guidelines^{1,2}, TBI is diagnosed by assessing the level of consciousness on the Glasgow Coma Scale (GCS) and analyzing data from computed tomography or magnetic resonance imaging. However, visual diagnostic methods are expensive and not always accessible, as they require patients to visit specialized centers. Also, existing clinical assessment scales, including the GCS, have limited sensitivity to hidden neuronal tissue damage.

As a result, a growing interest is being shown in objective laboratory markers capable of reflecting the

molecular and cellular processes behind neurotrauma. Among such biomarkers, particular attention is paid to interleukin-6 (IL-6), a pro-inflammatory cytokine that plays a key role in the pathogenesis of neuroinflammation and the activation of secondary injury cascades [4, 5]. Several studies show that IL-6 plasma levels correlate with TBI severity, brain edema development, and disease outcomes, especially within the first 24 h post-injury [6–8]. For example, the study by Hergenroeder et al. was one of the first works to demonstrate a correlation between serum IL-6 level and TBI severity [9]. Although these early studies used no machine learning tools, they laid the foundation for incorporating IL-6 into predictive models for TBI severity and outcomes [9].

However, in order to use IL-6 as a stratification tool in clinical practice, it is necessary to take into account its temporal dynamics in relation to other parameters [9, 10]. In this context, machine learning methods offer unique opportunities for constructing highly accurate predictive models capable of identifying nonlinear dependencies and determining the significance of individual time points. In particular, gradient boosting (XGBoost) and recurrent neural networks (LSTM), adapted for the analysis of time-series biomarkers, not only enhance

¹ Clinical guidelines "Mild Traumatic Brain Injury," 2016. URL: https://ruans.org/Text/Guidelines/mild_head_injury.pdf

² Clinical guidelines "Concussion," 2022. URL: <https://legalacts.ru/doc/klinicheskie-rekomendatsii-sotrasenie-golovnogo-mozga-utv-minzdravom-rossii/>

diagnostic accuracy but also ensure the interpretability of decisions through the use of SHAP (SHapley Additive exPlanations) analysis [11–13].

The present study addresses the development and validation of machine learning models for stratifying TBI severity based on serial IL-6 measurements, with a focus on their prognostic significance and potential for clinical integration into early diagnostic algorithms.

The study aims to develop and interpret a machine learning model for stratifying TBI severity based on the temporal dynamics of IL-6 plasma concentrations.

MATERIALS AND METHODS

Study Design and Population

The present study is a prospective cohort observation conducted to investigate the dynamics of IL-6 and its role in stratifying TBI severity in the early stages after traumatic impact. The study included 89 male athletes, with a mean age of 28.7 ± 5.3 years, who were actively engaged in contact sports (mixed martial arts and kudo). Inclusion criteria were as follows: a traumatic brain injury (confirmed clinically and through medical history), the absence of concomitant somatic pathology, and written informed consent to participate in the study.

The participants were stratified into three study groups by the clinical severity of TBI: a group with cerebral concussion ($n = 43$), a group with mild brain contusion ($n = 35$), and a group with moderate brain contusion ($n = 11$). This classification was based on the GCS score, duration of unconsciousness, and the presence of focal neurological symptoms.

Biological sample collection and analysis

For subsequent analysis of IL-6 level, venous blood was collected from the cubital vein into vacuum tubes containing ethylenediaminetetraacetic acid (K2EDTA) as an anticoagulant (VACUETTE® K2EDTA, Greiner Bio-One, Austria) at 3, 6, 12, and 24 h post-injury (t3, t6, t12, and t24). Blood samples were processed according to centrifugation protocols (3000 g; 10 min at 4°C), followed by plasma aliquoting and storage at -80°C until analysis.

IL-6 concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) with certified diagnostic kits (Accquant® HS-IL-6 ELISA, FineTest, China), manufactured in accordance with ISO 13485:2016 requirements. Laboratory analysis was performed at an accredited laboratory meeting the criteria of GOST ISO 15189:2015. Calibration curves were constructed based on standard samples, and quality control was ensured by using internal and external standards, which guaranteed an inter-assay variability of less than 10%.

Each IL-6 value was encoded as a time profile, enabling the use of the biomarker's dynamic characteristics in subsequent modeling. Missing values were assessed for their missingness mechanism and handled using multiple imputation algorithms. The complete dataset was normalized before being input into the machine learning models.

Definition of endpoints

In the stratification model, the primary clinical outcome variable (dependent variable) is TBI severity, determined based on a set of clinical criteria, including GCS scores, the presence and duration of unconsciousness, retrograde amnesia, as well as the severity of focal neurological symptoms. The participants were categorized into three diagnostic groups: cerebral concussion, mild brain contusion, and moderate brain contusion. The classification of injury severity was carried out in a neurological hospital setting according to standardized diagnostic protocols.

For the purpose of analysis, the categorical outcome was recoded into a binary variable: “non-severe TBI” (concussion + mild brain contusion) versus “severe TBI” (moderate brain contusion), with subsequent boundary validation based on predictive sensitivity modeling.

Machine Learning Tools

In order to implement a predictive tool for stratifying TBI severity based on time-series IL-6 concentrations, a combination of machine learning algorithms was applied. The primary model was built using XGBoost (Extreme Gradient Boosting) on tabular data, which included IL-6 values at four time points (3, 6, 12, and 24 h post-injury). The model hyperparameters were optimized using 5-fold cross-validation.

The interpretability of the model was assessed via the SHAP method, which provided a means to quantify the contribution of each time-specific IL-6 feature to the final classification. The greatest significance in predicting severe TBI outcomes was demonstrated by the IL-6 levels at 6 and 12 h post-injury (SHAP-value ≥ 0.37), which correlated with the phase of systemic inflammatory response.

In addition, an LSTM (Long Short-Term Memory) recurrent neural network was built for analysis of IL-6 time sequences (t3–t24) in order to model biomarker dynamics and predict outcomes. The input tensor had the shape $[n, 4, 1]$, where n is the number of patients. The model was trained using a supervised classification scheme, with a stratified split of the dataset into training and testing sets in an 80:20 ratio. The model's performance metrics were accuracy, F1-score, and area under the ROC curve. For model validation, a confusion matrix was also used to calculate sensitivity and specificity.

The machine learning methods used in this work expand and complement the capabilities of classical statistical tools. Classical methods (Kruskal–Wallis, Mann–Whitney, and ROC analysis) were employed to identify intergroup differences and assess the diagnostic significance of individual parameters. In contrast, machine learning algorithms (XGBoost, LSTM, and SHAP) were used to build accurate predictive models for individual patients in order to identify complex nonlinear relationships and temporal patterns in the analyzed parameters that cannot be detected using classical statistical models. The machine learning results were interpreted via the SHAP method, which provides a deeper and more intuitive interpretation of the contribution of predictors compared to standard regression coefficients. The combination of statistical approaches provided a means to identify significant differences and the diagnostic value of markers, while also ensuring an accurate, personalized outcome prediction.

Statistical analysis

All quantitative data were tested for normality of distribution using the Shapiro–Wilk test. Since a deviation from normal distribution was detected ($p \leq 0.05$), non-parametric methods were used to assess differences between groups: the Kruskal–Wallis test for comparing multiple groups and the Mann–Whitney test with Bonferroni correction for pairwise comparisons. Spearman's rank correlation coefficient (r_s) was used to assess the strength of association between variables.

The diagnostic accuracy of IL-6 biomarkers was assessed using area under the curve analysis (ROC analysis). In order to calculate sensitivity, specificity, and Youden's index ($J = Se + Sp - 1$, where Se is sensitivity and Sp is specificity), we used values corresponding to the optimal threshold criterion, which is determined by the maximum Youden's index. The significance of differences between ROC curves was evaluated employing the DeLong test. The interpretation of AUC (area under the curve) values was carried out in accordance with recommendations for measuring the accuracy of diagnostic systems [14]: AUC 0.7–0.8 — fair accuracy; 0.8–0.9 — good; >0.9 — excellent.

In order to develop a model for stratifying the severity of traumatic brain injury, gradient boosting (XGBoost) was applied. The training and test sets were formed in a 70:30 ratio with stratification (stratified split) to ensure balanced representation of classes. Model hyperparameters were selected using stratified 5-fold cross-validation, in which the training set was sequentially divided into five equal subsets while maintaining class proportions in each. In each iteration, four subsets were used for model training, and one subset was used for quality assessment. Hyperparameter optimization was performed by minimizing the log-loss. The quality of the final model was evaluated on the test set using the following

metrics: area under the ROC curve (AUC-ROC), precision, recall, and F1-score. The contributions of features to model predictions were interpreted via the SHAP method in its specialized implementation for tree ensembles (TreeSHAP).

For temporal prediction and patient stratification, a model based on an LSTM recurrent neural network was also implemented. Time series were generated from four measurement points (3, 6, 12, and 24 h) and were normalized using the z-score scale. Training was performed using the Adam optimizer and the categorical cross-entropy loss function. Early stopping, which relies on the loss value on the validation set, was used to combat overfitting. The accuracy of the model was compared with baseline algorithms (logistic regression and XGBoost).

All statistical calculations and visualizations were performed in the R environment (version 4.3.2) using the following packages: tidyverse, ggplot2, pROC, xgboost, SHAPforxgboost, keras, caret, and rstatix. Differences were considered statistically significant at $p \leq 0.05$, with adjustments for multiple comparisons where appropriate.

RESULTS AND DISCUSSION

A time profile analysis of IL-6 concentrations (t3, t6, t12, and t24) revealed significant intergroup differences depending on the clinical severity of TBI. Figure 1 presents the distributions of IL-6 values, indicating medians and interquartile ranges for the three cohorts: cerebral concussion, mild brain contusion, and moderate brain contusion.

The most pronounced differences between groups were noted at 6 and 12 h post-injury. According to the Kruskal–Wallis test, a statistically significant difference in IL-6 concentrations was observed at these time points between the three severity categories ($p < 0.001$). Conversely, the 3-h and 24-h time points did not reach the level of statistical significance after the Bonferroni correction ($p > 0.05$).

In pairwise comparisons (Mann–Whitney test), IL-6 concentrations in moderate TBI patients exceeded the corresponding values in patients with mild brain contusion and concussion by an average of 1.8–2.3 times at 6 and 12 h post-injury, indicating the presence of a diagnostically relevant window of inflammatory activity within 3–12 h after trauma.

Spearman's correlation analysis revealed a positive relationship between IL-6 level and severity in these time intervals ($r_s = 0.81$; $p < 0.001$). The most informative indicators were found in samples obtained at 6 and 12 h post-injury, which is consistent with the phase of systemic inflammatory response and justifies their inclusion in prognostic stratification algorithms (Fig. 2).

In order to develop a tool for stratifying TBI severity, an XGBoost model was built using IL-6 concentration

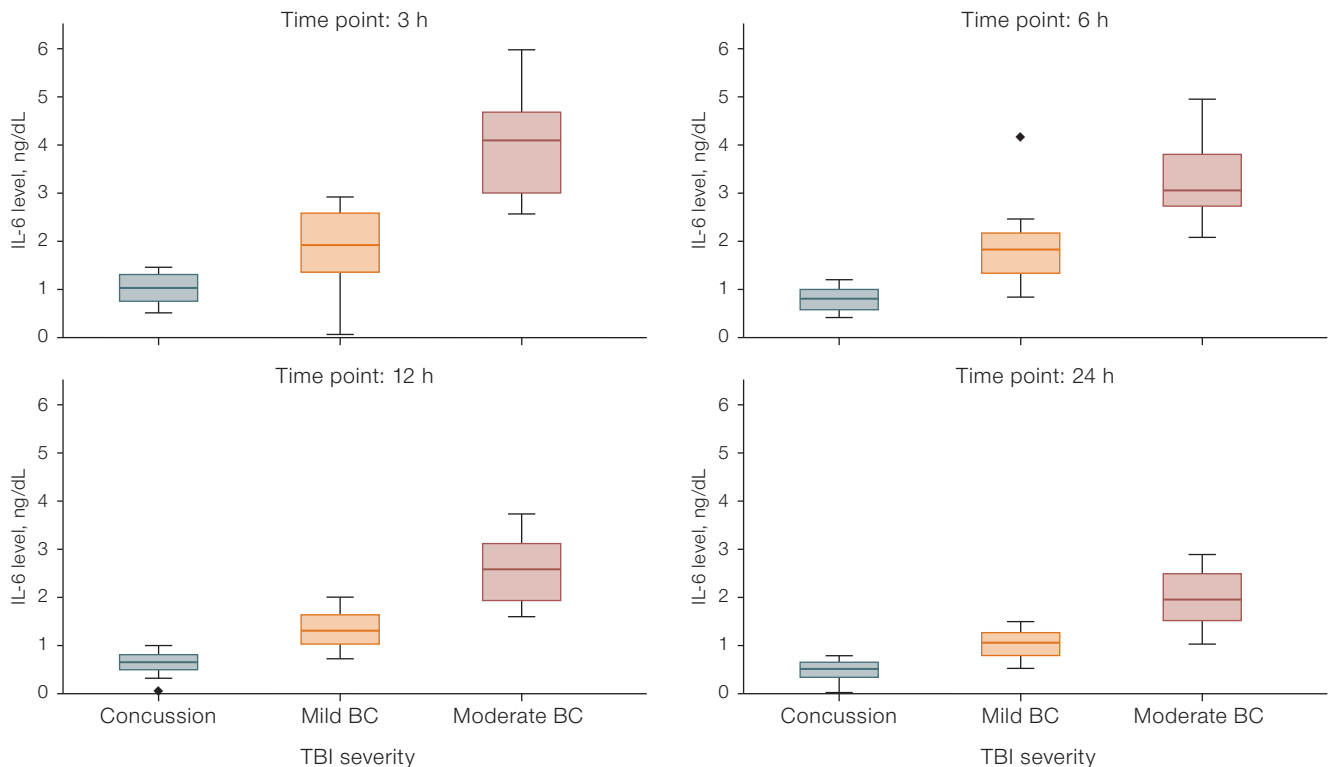


Figure prepared by the authors based on their own data

Fig. 1. Interleukin-6 level distribution in groups with concussion, mild brain contusion, and moderate brain contusion at 3, 6, 12, and 24 h post-injury: ♦ — outliers beyond the interquartile ranges (i.e., observations in the dataset that significantly deviate from the other values); BC — brain contusion; TBI — traumatic brain injury

values at four time points as input parameters: 3, 6, 12, and 24 h post-injury.

Each row in the graph corresponds to one time point of IL-6 measurement (3, 6, 12, and 24 h post-injury), and the horizontal axis represents the SHAP value, i.e., the quantitative contribution of each feature to the final model prediction. Points to the right of the zero line (positive SHAP values) indicate an increased risk of being classified as severe TBI, while points to the left indicate a decreased risk. The color scale (from yellow to purple) represents the effect on the model response: yellow points correspond to samples with a low effect on the response, purple points to those with a significant effect. The pronounced shift in the distribution of points for IL-6_{6h} (IL6_{t6}) and IL-6_{12h} (IL6_{t12}) into the positive region (to the right), with maximum SHAP values ≥ 0.37 , demonstrates a significant contribution and correlates with the phase of systemic inflammatory response in TBI.

A feature importance analysis based on the reduction in classification accuracy and the Gini index showed that the IL-6 values obtained at 6 and 12 h post-injury made the maximum contribution to the classification of severe TBI forms (Fig. 3). These time points were also identified as key in the non-parametric analysis ($H = 31.77$; $p < 0.001$ and $H = 28.91$; $p < 0.001$), indicating the reproducibility of the result across independent

methodological platforms. The greatest contribution to classification is made by the IL-6 values recorded at 3 and 6 h post-injury, which confirms their diagnostic significance (Fig. 3).

The diagnostic accuracy of the model, measured by the AUC-ROC, amounted to 0.92 in a multi-class approach, indicating high prediction reliability. The SHAP analysis confirmed that at 6 and 12 h post-injury, the IL-6 values had a decisive influence on the classification of moderate TBI cases. For predicting the moderate form, an IL-6 threshold of >12.5 pg/mL was determined at these time points.

The modeling results underscore the prognostic significance of inflammatory response within the window between 6 and 12 h after injury. In clinical diagnostic algorithms, these biomarkers could enhance the sensitivity and specificity of stratification models and, consequently, increase the proportion of complications detected at early stages. The interpretability is ensured by means of SHAP visualization (Fig. 2), which provides grounds for considering the model to be clinically relevant and suitable for validation on external cohorts.

A comparison of predictive capabilities possessed by the machine learning algorithms revealed the superiority of non-linear ensemble models in classifying TBI severity based on time-series IL-6 concentrations. Within a

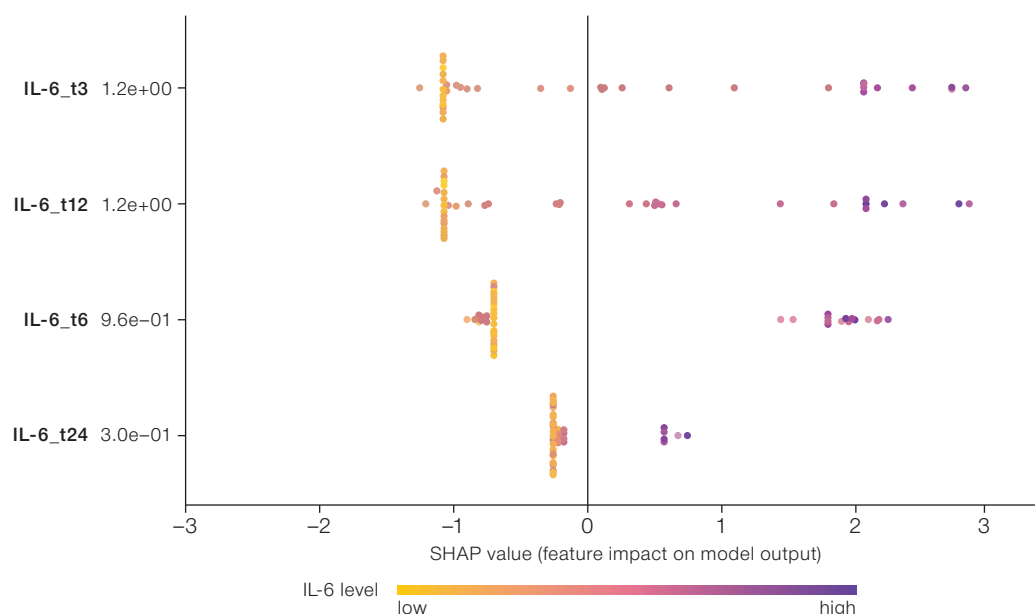


Figure prepared by the authors based on their own data

Fig. 2. SHAP analysis of the contribution of interleukin-6 time points to the XGBoost model for traumatic brain injury stratification: the color scale represents the interleukin-6 level, from low (yellow) to high (purple)

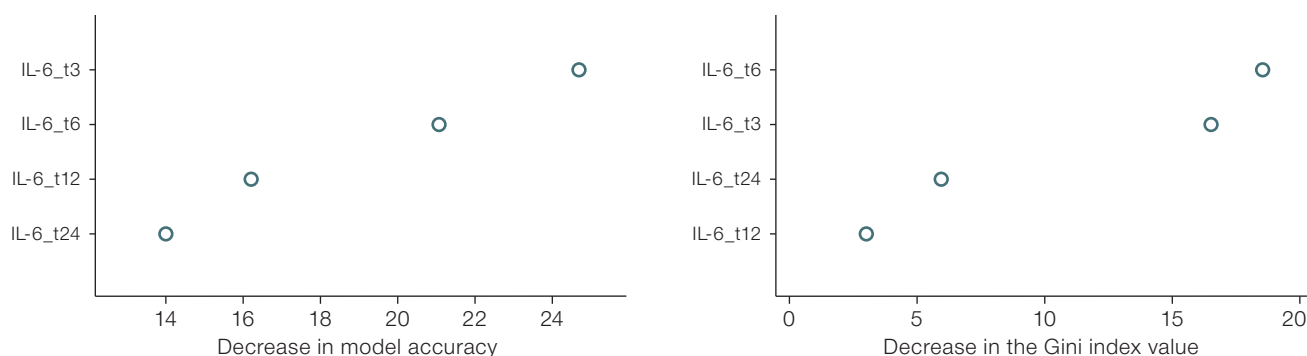


Figure prepared by the authors based on their own data

Fig. 3. Feature importance plots calculated based on the Random Forest algorithm: the left panel shows a decrease in model accuracy when the corresponding feature is excluded; the right panel shows a decrease in the Gini index value

stratified dataset built on the levels of neuroinflammatory response, four measurement points were used: 3, 6, 12, and 24 h.

The gradient boosting model (XGBoost) demonstrated the highest diagnostic accuracy ($AUC = 0.92$), which surpassed that of random forest ($AUC = 0.88$) and regularized logistic regression ($AUC = 0.84$). The differences between the models were statistically confirmed by the DeLong test ($\chi^2 = 12.41$; $p = 0.002$). The optimal sensitivity and specificity for XGBoost reached 87% and 84%, respectively, at the maximum Youden's index.

The most significant contribution to the model output was made by IL-6 concentrations obtained at 6 and 12 h post-injury, which is consistent with the correlation analysis and non-parametric comparisons. These time

points form a diagnostically sensitive window for stratifying TBI severity.

Summary data on the accuracy metrics of all models are presented in the table.

The presented data support the application of a comprehensive approach, which is based on multi-time IL-6 profiles, to stratifying neurotrauma severity in athletes from contact sports.

In order to analyze the IL-6 concentration time series and classify TBI severity, an LSTM model was implemented (Fig. 3). The input data consisted of sequences of IL-6 values obtained at 3, 6, 12, and 24 h post-injury. The model architecture was adapted to the tensor dimensions $[n, 4, 1]$, where n is the number of participants.

The training was conducted using a supervised classification principle with an 80:20 dataset split, while

Table. Model accuracy metrics and the contribution of IL-6 sampling time to the classification of traumatic brain injury severity

Model	AUC [95% CI]	Sensitivity (%)	Specificity (%)	Youden's index	Most significant IL-6 sampling time, h
XGBoost	0.92 [0.87–0.96]	87	84	0.71	6; 12
Random Forest	0.88 [0.82–0.93]	82	80	0.62	6
Logistic Regression	0.84 [0.77–0.89]	78	76	0.54	12

Table compiled by the authors based on their own data

Note: AUC — Area Under the Curve; XGBoost (eXtreme Gradient Boosting) — machine learning model based on gradient boosting algorithms; IL-6 — interleukin-6.

maintaining the stratified distribution by severity levels. The model showed an average classification accuracy of 84.5%, and the F1-score for the group with moderate brain contusion reached 0.91. This indicates a high ability of the model to correctly identify the most severe cases, taking into account the temporal dynamics of the inflammatory biomarker.

A sensitivity analysis to feature exclusion revealed the key role of IL-6 levels measured at 6 h post-injury. Removal of this feature led to an AUC decrease of 0.08, confirming its critical importance in prediction. The visualized model outputs showed that the 6–12 h temporal pattern makes the greatest contribution to group differentiation, correlating with the previously identified window of systemic inflammatory response.

The obtained results suggest that recurrent neural networks are a promising tool for interpreting temporal biomolecular signals in TBI stratification. Such models can be implemented with the use of sequential biomarkers and standardized time points, which ensures not only high accuracy but also the clinical applicability of the algorithm.

The study results indicate a high prognostic value of IL-6 levels in stratifying TBI severity, particularly within the 6–12 h post-injury interval. This aligns with the established pathophysiological phases of neuroinflammatory response, during which IL-6 participates in cascade activation, leading to secondary neuronal and glial damage.

A comparison with the results of a prospective TRACK-TBI study shows the comparable significance of IL-6, glial fibrillary acidic protein (GFAP), and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) in predicting outcomes in mild to moderate TBI [15]. Unlike GFAP and UCHL1, which primarily reflect structural damage to astrocytes and axons, IL-6 helps characterize the systemic inflammatory response, especially in early post-injury stages [4, 5, 7, 16]. Papa et al. show that GFAP/UCHL1 levels can predict the presence of intracranial abnormalities on computed tomography scans but do not cover the dynamic spectrum of immune activation [17–19].

In this study, the IL-6 levels at 6 and 12 h post-injury demonstrated the highest prognostic significance (SHAP

value ≥ 0.37 ; $p < 0.001$), making a key contribution to the XGBoost model (Figs. 2, 3). This is consistent with the concept of “systemic vulnerability interval” described by Yue et al. [20], according to which early cytokine cascade activation is crucial in determining prognosis and injury outcomes.

The performed comparative analysis of the algorithms indicates the advantage of non-linear models, including XGBoost (AUC = 0.92) and LSTM (F1 = 0.91 for moderate brain contusion), over logistic regression and random forest (Table). The model interpretation through SHAP analysis confirms the key role of the 6 and 12-h time points in classifying severity. This makes IL-6 not only diagnostically significant but also a clinically applicable biomarker for monitoring in the subacute period.

The presence of a 6–12 h diagnostic window is also reflected in the visual distributions of IL-6 concentrations (Fig. 1), where statistically significant differences were observed at these time points between all values in the severity groups ($H = 31.77$; $p < 0.001$ and $H = 28.91$; $p < 0.001$, respectively). Thus, these time points can be considered critical in developing laboratory monitoring protocols and making clinical decisions.

Noteworthy is that, unlike most existing studies, the present modeling was based on a strictly stratified cohort with serial measurements, which increased the accuracy of predictions. However, the use of only one biomarker (IL-6) and the relatively small size of the subgroup with moderate brain contusion ($n = 11$) still impose limitations, requiring subsequent external validation on independent samples.

The conducted study confirms that the post-TBI interval between 6 and 12 h has the highest diagnostic value in assessing injury severity based on plasma IL-6 concentrations. It was during this period that the subgroup of patients with moderate brain contusion exhibited the maximum median IL-6 levels, which significantly exceeded those in the groups with mild injury and concussion (Fig. 1). The statistical significance of differences at 6 and 12 h post-injury was confirmed by the non-parametric Kruskal–Wallis test: $H = 31.77$;

$p < 0.001$ for the 6 h time point and $H = 28.91$; $p < 0.001$ for the 12 h time point.

The prognostic value of these time points was further confirmed within the construction of machine learning models. According to gradient boosting (XGBoost), IL-6 concentrations at 6 and 12 h post-injury had the highest importance values both in terms of the decrease in model accuracy and the Gini index (Fig. 3). Using SHAP analysis to interpret the model, we found that these two temporal features had the most significant effect on the model's predictions (maximum SHAP values ≥ 0.37). This approach ensured a high level of algorithm explainability, which is critical for application in clinical practice.

Along with XGBoost, the LSTM model adapted to the time-series biomarker data demonstrated high accuracy. Despite the small sample size ($n = 89$), it achieved an F1-score of 0.91 in the classification of TBI severity. This indicates the potential of LSTM neural networks in multi-class stratification and monitoring.

However, the methodology has several limitations. First, the study relies exclusively on IL-6 levels, without the use of other inflammatory, glial, and axonal biomarkers. This limits the potential for constructing multi-biomarker models, whose promise has been demonstrated in large cohort studies. Second, the sample size ($n = 89$) does not allow for robust external validation of the models, especially within subgroups where patients with moderate brain contusion account for less than 15% of the total cohort. Finally, the IL-6 levels were measured using only one analytical method (ELISA), which limits data reproducibility in interlaboratory settings.

Nevertheless, plasma IL-6 levels possess high diagnostic accuracy in predicting TBI severity, particularly within the diagnostically sensitive post-injury window between 6 and 12 h. This conclusion is confirmed not only via statistical methods but also by interpretable machine learning models, which provides a foundation for incorporating IL-6 into digital protocols for personalized monitoring and decision-making in sports medicine and military medical practice.

Clinical and regulatory aspects of implementing intelligent models in neurocritical care

Current research on TBI identification extensively explores machine learning approaches [21, 22], and machine learning algorithms are actively used to create predictive models for TBI patient outcomes. For instance, in the study by Cao et al. [23], XGBoost and SHAP were used to develop and validate a predictive model for in-hospital mortality in patients with isolated severe TBI and to identify the most influential predictors. This model proved to be highly accurate in predicting in-hospital mortality within the first five days based on injury severity, admission GCS score, and patient age.

For the prediction of GCS score dynamics in TBI patients, Nayeby et al. propose a model based on a deep

recurrent neural network (including LSTM). This study showed that training a model on time-series data from TBI patients can be informative and improve prediction accuracy. The most important features were derived from the recurrent neural network model; their values show a clear trend for favorable and unfavorable outcomes. This study demonstrates the amount of information that can be obtained from time-series data for more accurate TBI prediction [24].

Using artificial intelligence algorithms to stratify TBI severity opens up prospects for integrating intelligent clinical decision support systems (CDSS) into the practice of neurocritical care. Validated models built on data regarding IL-6 concentrations and other biomarkers provide a means to rapidly assess the likelihood of developing adverse neurological outcomes and determining injury severity in the early stages post-incident.

In order to ensure regulatory compatibility of the proposed solutions, compliance with international standards for the reliability and interpretability of AI/ML approaches is of particular importance. Specifically, the structure of the developed algorithms and their logic align with the principles of ISO/IEC 23053:2022. This standard provides a framework for describing AI systems incorporating machine learning, with provisions for ensuring transparency, robustness, and ethical acceptability of digital solutions. Adherence to such a framework is especially critical when algorithms are applied in contexts of clinical uncertainty and under life-threatening conditions.

A key condition for the clinical acceptability of intelligent models is their interpretability by medical professionals. This study utilized explainable AI (XAI) methods, including SHAP value analysis, which enables a personalized assessment of the contribution of each feature (specifically, IL-6 levels at 6 or 12 h post-injury) to the final model output. This allows an expert physician to verify the result and make informed clinical decisions.

Noteworthy is that to be used in clinical settings, algorithms must be validated on independent samples. In this study, the generalizability of results was limited by the use of a single cohort. The transition to clinical application would require multicenter studies with external datasets to confirm the model's robustness across various populations and laboratory settings. Only with reproducible accuracy ($AUC > 0.85$) and a controlled error rate (as per ISO 14971 [8]) can such algorithms be included in regulated patient management protocols for TBI.

CONCLUSION

The study developed a predictive model that uses gradient boosting and recurrent neural networks to analyze temporal inflammatory biomarkers within the first 24 h after TBI.

The conducted time profile analysis of IL-6 during the post-traumatic period in TBI patients revealed the

significance of 6 and 12-h post-injury time points in clinical severity stratification. The measured IL-6 concentrations demonstrated a stable association with clinical assessment and significant differences between groups according to non-parametric analysis results ($H = 31.77$; $p < 0.001$) and correlation coefficients with neurological status scales ($r_s = 0.81$; $p < 0.001$).

The developed machine learning models — XGBoost and LSTM recurrent neural networks — provided high stratification accuracy, which was confirmed by the area under the ROC curve values ($AUC > 0.90$) and robustness to overfitting during cross-validation. The most informative features in these models were the IL-6 levels within the specified critical intervals.

The SHAP feature importance method helped determine the predictive contribution of each time marker in the model. This approach provided a means to preserve the clinical interpretability of the model and ensure the physician's participation in the decision-making process.

The scientific significance of this study lies in the identification of critical time points for IL-6 measurement (6 and 12 h), which could potentially enable the standardization of future research. The diagnostically significant IL-6 concentrations identified in the early period correlate with TBI outcomes, confirming the hypothesis about the critical role of an early and exaggerated neuro-inflammatory response in the pathogenesis of secondary brain injury. This shifts the focus of attention to the very earliest post-traumatic processes. Moreover, the new data can be used as input parameters for creating machine learning models.

In practice, the obtained results can be used as follows: additional diagnostic methods alongside GCS assessment and CT data; a decision-making tool in the absence of radiological diagnostic methods; a criterion for personalized therapy; markers of recovery. Furthermore, the study results will allow patients to be classified not only by the GCS score but also by early inflammatory response in the testing of new neuroprotective agents, thereby increasing the precision of research.

Early TBI diagnosis provides a means to identify critical patients, determine patient management strategy, and predict the course of rehabilitation. In this study, we quantitatively assessed the contribution of each time-specific IL-6 measurement to the prediction of TBI severity with the use of machine learning algorithms (including SHAP analysis), which ensured the clinical interpretability of the model and its potential for integration into a personalized system for neurotrauma diagnosis.

The study results advance laboratory TBI diagnostics that incorporates machine learning. In the clinical settings of emergency departments, neuro-intensive care units, and neurosurgery departments, such approaches will enable early diagnosis and assist in medical decision-making.

Thus, the obtained data demonstrate the potential for integrating intelligent models into personalized medical care systems for TBI patients. The use of inflammatory molecular markers in combination with algorithmic analysis of time-series data allows for more accurate prediction of neurological outcomes and the development of individualized strategies for post-traumatic rehabilitation.

References

- Butkova TV, Malsagova KA, Nakhod VI, Petrovskiy DV, Izotov AA, Balakin EI, et al. Candidate Molecular Biomarkers of Traumatic Brain Injury: A Systematic Review. *Biomolecules*. 2024;14(10):1283. <https://doi.org/10.3390/biom14101283>
- VanItallie TB. Traumatic brain injury (TBI) in collision sports: Possible mechanisms of transformation into chronic traumatic encephalopathy (CTE). *Metabolism*. 2019;100S:153943. <https://doi.org/10.1016/j.metabol.2019.07.007>
- Gerber KS, Alvarez G, Alamian A, Behar-Zusman V, Downs CA. Biomarkers of Neuroinflammation in Traumatic Brain Injury. *Clinical Nursing Research*. 2022;31(7):1203–18. <https://doi.org/10.1177/10547738221107081>
- Ichwan K, Gazali S, Suherman S, Desiana D, Nurjannah N. Plasma interleukin 6 as an outcome predictor of traumatic brain injury patients. *Narra J*. 2023;3(3):e234. <https://doi.org/10.52225/narra.v3i3.234>
- Li G, Liu H, He Y, Hu Z, Gu Y, Li Y, et al. Neurological Symptoms and Their Associations With Inflammatory Biomarkers in the Chronic Phase Following Traumatic Brain Injuries. *Frontiers in Psychiatry*. 2022;13:895852. <https://doi.org/10.3389/fpsy.2022.895852>
- Malik S, Alnaji O, Malik M, Gambale T, Farrokhyar F, Rathbone MP. Inflammatory cytokines associated with mild traumatic brain injury and clinical outcomes: a systematic review and meta-analysis. *Frontiers in Neurology*. 2023;14:1123407. <https://doi.org/10.3389/fneur.2023.1123407>
- Ooi SZY, Spencer RJ, Hodgson M, Mehta S, Phillips NL, Preest G, et al. Interleukin-6 as a prognostic biomarker of clinical outcomes after traumatic brain injury: a systematic review. *Neurosurgical Review*. 2022;45:3035–54. <https://doi.org/10.1007/s10143-022-01827-y>
- Yang J, Ran M, Li H, Lin Y, Ma K, Yang Y, et al. New insight into neurological degeneration: Inflammatory cytokines and blood-brain barrier. *Frontiers in Molecular Neuroscience*. 2022;15:1013933. <https://doi.org/10.3389/fnmol.2022.1013933>
- Hergenroeder GW, Moore AN, McCoy JP, Samsel L, Ward NH, Clifton GL, et al. Serum IL-6: a candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury. *Journal of Neuroinflammation*. 2010;7:19. <https://doi.org/10.1186/1742-2094-7-19>
- Helmy A, Carpenter KLH, Menon DK, Pickard JD, Hutchinson PJA. The Cytokine Response to Human Traumatic Brain Injury: Temporal Profiles and Evidence for Cerebral Parenchymal Production. *Journal of Cerebral Blood Flow & Metabolism*. 2010;31(2):658–70. <https://doi.org/10.1038/jcbfm.2010.142>
- Lundberg S, Lee S-I. A Unified Approach to Interpreting Model Predictions. NIPS'17: *Proceedings of the 31st International Conference on Neural Information Processing*

- Systems. 2017;arXiv:1705.07874v2.
<https://doi.org/10.48550/ARXIV.1705.07874>
12. Che Z, Purushotham S, Cho K, Sontag D, Liu Y. Recurrent Neural Networks for Multivariate Time Series with Missing Values. *Scientific Reports*. 2018;8:6085.
<https://doi.org/10.1038/s41598-018-24271-9>
 13. Mekkodathil A, El-Menyar A, Naduvilekandy M, Rizoli S, Al-Thani H. Machine Learning Approach for the Prediction of In-Hospital Mortality in Traumatic Brain Injury Using Bio-Clinical Markers at Presentation to the Emergency Department. *Diagnostics*. 2023;13(15):2605.
<https://doi.org/10.3390/diagnostics13152605>
 14. Swets JA. Measuring the Accuracy of Diagnostic Systems. *Science*. 1988;240(4857):1285–93.
<https://doi.org/10.1126/science.3287615>
 15. Gardner RC, Puccio AM, Korley FK, Wang KKW, Diaz-Arrastia R, Okonkwo DO, et al. Effects of age and time since injury on traumatic brain injury blood biomarkers: a TRACK-TBI study. *Brain Communications*. 2023;5(1):fcac316.
<https://doi.org/10.1093/braincomms/fcac316>
 16. Reyes J, Spitz G, Major BP, O'Brien WT, Giesler LP, Bain JWP, et al. Utility of Acute and Subacute Blood Biomarkers to Assist Diagnosis in CT-Negative Isolated Mild Traumatic Brain Injury. *Neurology*. 2023;101(20):e1992–2004.
<https://doi.org/10.1212/WNL.000000000000207881>
 17. Papa L, Brophy GM, Welch RD, Lewis LM, Braga CF, Tan CN, et al. Time Course and Diagnostic Accuracy of Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 in a Large Cohort of Trauma Patients With and Without Mild Traumatic Brain Injury. *JAMA Neurology*. 2016;73(5):551–60.
<https://doi.org/10.1001/jamaneurol.2016.0039>
 18. Papa L, Ladde JG, O'Brien JF, Thundiyil JG, Tesar J, Leech S, et al. Evaluation of Glial and Neuronal Blood Biomarkers Compared With Clinical Decision Rules in Assessing the Need for Computed Tomography in Patients With Mild Traumatic Brain Injury. *JAMA Network Open*. 2022;5(3):e221302.
<https://doi.org/10.1001/jamanetworkopen.2022.1302>
 19. Papa L, McKinley WI, Valadka AB, Newman ZC, Nordgren RK, Pramuka PE, et al. Diagnostic Performance of GFAP, UCH-L1, and MAP-2 Within 30 and 60 Minutes of Traumatic Brain Injury. *JAMA Network Open*. 2024;7(9):e2431115.
<https://doi.org/10.1001/jamanetworkopen.2024.31115>
 20. Yue JK, Kobeissy FH, Jain S, Sun X, Phelps RRL, Korley FK, et al. Neuroinflammatory Biomarkers for Traumatic Brain Injury Diagnosis and Prognosis: A TRACK-TBI Pilot Study. *Neurotrauma Reports*. 2023;4(1):171–83.
<https://doi.org/10.1089/neur.2022.0060>
 21. Malhotra AK, Shakil H, Smith CW, Huang YQ, Kwong JCC, Thorpe KE, et al. Predicting outcomes after moderate and severe traumatic brain injury using artificial intelligence: a systematic review. *NPJ Digital Medicine*. 2025;8:373.
<https://doi.org/10.1038/s41746-025-01714-y>
 22. Courville E, Kazim SF, Vellek J, Tarawneh O, Stack J, Roster K, et al. Machine learning algorithms for predicting outcomes of traumatic brain injury: A systematic review and meta-analysis. *Surgical Neurology International*. 2023;14:262.
https://doi.org/10.25259/SNI_312_2023
 23. Cao Y, Forssten MP, Sarani B, Montgomery S, Mohseni S. Development and Validation of an XGBoost-Algorithm-Powered Survival Model for Predicting In-Hospital Mortality Based on 545,388 Isolated Severe Traumatic Brain Injury Patients from the TQIP Database. *Journal of Personalized Medicine*. 2023;13(9):1401.
<https://doi.org/10.3390/jpm13091401>
 24. Nayeibi A, Tipirneni S, Foreman B, Ratcliff J, Reddy CK, Subbian V. Recurrent Neural Network based Time-Series Modeling for Long-term Prognosis Following Acute Traumatic Brain Injury. *AMIA Annual Symposium Proceedings*. 2022;2021:900–9.

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INVESTIGATION OF BIOTRANSFORMATION PROCESSES AND PHARMACOLOGICAL ACTIVITY OF VIOLURIC ACIDS AND THEIR METABOLITES IN *IN VIVO* EXPERIMENTS

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Introduction. 5-Hydroxyiminobarbituric (violuric) acid and its derivatives exhibit pronounced anti-hypoxic, hepatoprotective, cytoprotective, actoprotective, and other properties, making this group of compounds a promising field for pharmaceutical research. Data on the metabolism of violuric acids (VAs) are of significant practical and theoretical importance for the tracking of pharmacokinetics and substance distribution within the organism, in which process metabolites serve as markers of biochemical processes involving endogenous substrates.

Objective. To determine the structure of violuric acid metabolites and perform their quantitative assessment in an *in vivo* experiment.

Materials and methods. The studied substances (VAs) and their metabolites (purpuric acids) were synthesized at the Golikov Research Center of Toxicology. Their structure and purity were confirmed by nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), and spectrophotometry (SP). Metabolism and anti-hypoxic activity were studied using a model of hemic hypoxia induced by a lethal dose of sodium nitrite in outbred male white rats. Solutions of violuric acids for *in vitro* studies and administration to animals were prepared in distilled water with the addition of tris(oxyethyl)aminomethane (TRIS), while purpurates in the form of salts were dissolved in distilled water. The dosages of the test substances for rats ranged from 50–75 mg/kg for intraperitoneal administration and 50–100 mg/kg for intragastric administration. The reference substance (amtirole succinate) was administered to animals at a dose of 100 mg/kg. A 0.9% sodium chloride solution, administered at a volume of 1 mL per animal, was used as a control. Quantitative analysis of the substances and their metabolites in biological media was performed by HPLC with SP detection.

Results. It was established that the studied substances (violuric acid, 2-thiovioluric acid, 1-butylvioluric acid, and 1-(4-bromophenyl)violuric acid) are metabolized in the animal organism to form the corresponding derivatives of purpuric acid (purpurates), whose structure was confirmed by counter synthesis. Violuric acids and their metabolites are primarily excreted in the urine. It was demonstrated that 1-butylvioluric acid and its metabolite *N,N'*-dibutylpurpuric acid exhibit pronounced anti-hypoxic activity in the model of acute sodium nitrite poisoning, preventing mortality in 100% of animals, whereas the reference antihypoxant amtirole only prolongs survival time (by 23%).

Conclusions. The formation of purpurates is a characteristic pathway of metabolic transformation for the violuric acid scaffold. These metabolites exhibit pronounced activity and, in all likelihood, can contribute to the overall biological effect of violuric acids.

Keywords: violuric acid; derivatives; metabolism; purpuric acids; rats; antihypoxants; HPLC; amtirole

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ИЗУЧЕНИЕ ПРОЦЕССОВ БИОТРАНСФОРМАЦИИ И ФАРМАКОЛОГИЧЕСКОЙ АКТИВНОСТИ ВИОЛУРОВЫХ КИСЛОТ И ИХ МЕТАБОЛИТОВ В ЭКСПЕРИМЕНТАХ *IN VIVO*

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Введение. 5-Оксииминобарбитуровая (виолуровая) кислота и ее производные проявляют выраженные антигипоксические, гепатопротекторные, цитопротекторные, актопротекторные и другие свойства, что делает эту группу веществ перспективным полем для фармацевтических изысканий. Данные о метаболизме виолуровых кислот (ВК) представляют большую практическую и теоретическую значимость, позволяя отслеживать фармакокинетику и распределение вещества в организме, причем метаболиты служат маркерами биохимических процессов, протекающих при участии эндогенных субстратов.

Цель. Установление структуры продуктов метаболизма виолуровых кислот и их количественная оценка в эксперименте *in vivo*.

Материалы и методы. Исследуемые вещества (ВК) и продукты их метаболизма (пурпуровые кислоты) были синтезированы на базе Научно-клинического центра токсикологии им. С.Н. Голикова Федерального медико-биологического агентства, их структура и чистота подтверждены методами ядерного магнитного резонанса (ЯМР), высокоэффективной жидкостной хроматографии (ВЭЖХ)

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и спектрофотометрии (СФ). Метаболизм и антигипоксическую активность изучали на модели гемической гипоксии, вызванной смертельной дозой нитрита натрия, на беспородных белых крысах-самцах. Растворы виолуровых кислот для исследований *in vitro* и введения животным готовили в дистиллированной воде с добавкой трис(оксиметил)аминометана (ТРИС), а пурпураты, используемые в форме солей, растворяли в дистиллированной воде. Дозировки исследуемых веществ для крыс составляли 50–75 мг/кг при внутривенном и 50–100 мг/кг при внутрижелудочном введении. Эталонное вещество (амтизол сукцинат) вводили животным в дозе 100 мг/кг. В качестве контроля использовали раствор хлорида натрия 0,9%, который вводили в объеме 1 мл на животное. Количественный анализ веществ и их метаболитов в биосредах осуществлен методом ВЭЖХ с СФ-детектированием.

Результаты. Установлено, что исследуемые вещества (виолуровая, 2-тиовиолуровая, 1-бутилвиолуровая и 1-(4-бромфенил)виолуровая кислоты) в организме животных метаболизируются с образованием соответствующих производных пурпуровой кислоты (пурпуратов), структура которых была доказана встречным синтезом. ВК и продукты их метаболизма выводятся преимущественно с мочой. Показано, что 1-бутилвиолуровая кислота и ее метаболит *N,N'*-дибутилпурпуровая кислота на модели острого отравления NaNO_2 обладают выраженной антигипоксической активностью, предотвращая гибель 100% животных, тогда как эталонный антигипоксикант Амтизол лишь продлевает время жизни (на 23%).

Выводы. Образование пурпуратов является характерным направлением метаболической трансформации скаффолда виолуровых кислот. Данные метаболиты обладают выраженной активностью и, по всей видимости, могут участвовать в формировании общего биологического эффекта виолуровых кислот.

Ключевые слова: виолуровая кислота; производные; метаболизм; пурпуровые кислоты; крысы; антигипоксиканты; ВЭЖХ; амтизол

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INTRODUCTION

Antihypoxants, antioxidants, adaptogens, actoprotectors, and other pharmacological agents that enhance the body's adaptive capabilities play a significant role in modern pharmacology. Drugs from these pharmaceutical classes — primarily antihypoxants — are widely used in various medical fields, such as surgical practice [1], cardiology [2], neurology [3, 4], sports and extreme medicine [5], psychiatry [6], in cases of traumatic brain injury and cerebrovascular disorders [7, 8], treatment of liver intoxications [9], and many other areas [10]. Consequently, the development of new anti-hypoxic drugs and the search for novel approaches in the therapy of hypoxic conditions remain highly relevant [11].

Priority directions in the search for antihypoxants focus on original groups of synthetic heterocyclic compounds, among which are found substances capable of engaging additional mechanisms to enhance the body's adaptive reserve. In this regard, 5-hydroxyiminobarbituric acids, also referred to as violuric acids (VAs), represent a group of heterocyclic oximes offering promising pharmacological properties [12].

The simplest derivative of this series is the unsubstituted violuric acid (VA1), whose structure is presented in

Figure 1. This substance was investigated in a previous study,¹ in which its high antidotal efficacy in models of hemic hypoxia induced by carbon monoxide and methemoglobin-forming poisons (sodium nitrite, aniline, etc.) was established, surpassing standards such as unithiol, amtizole, gutimine, and methylene blue. VA1 also demonstrated good results in models of hypobaric and hypercapnic hypoxia. Another derivative of this series, 2-thiovioluric acid (VA2), similarly exhibited antidotal activity in nitrite-induced hemic hypoxia. Furthermore, VA2 showed a pronounced protective effect in a model of nitric oxide-induced pulmonary edema.² 1-Butylvioluric acid (VA3), used in the form of a zinc complex, demonstrated a protective effect in carbon monoxide poisoning, surpassing the known drug acizol, as well as showing a therapeutic effect in animals poisoned with neurotoxic agents [12].

For VA1 and its analogs, certain antioxidant and membrane-stabilizing properties, as well as the ability to increase hemoglobin stability *in vitro*, have been noted at the biochemical level [12, 13].³ In addition to the aforementioned types of activity, various derivatives within the VA series have demonstrated hepatoprotective, antimicrobial, antiviral, analgesic, tranquilizing, and other properties [14]. It is worth noting separately that

¹ Burbello AT. Barbituric Acid Derivatives — A New Class of Compounds for the Prevention and Treatment of Nitrocompound Poisonings. Abstract of Doctoral Dissertation (Dr. Med. Sci.). St. Petersburg, 1991 (In Russ.).

² Ibid.

³ Ibid.

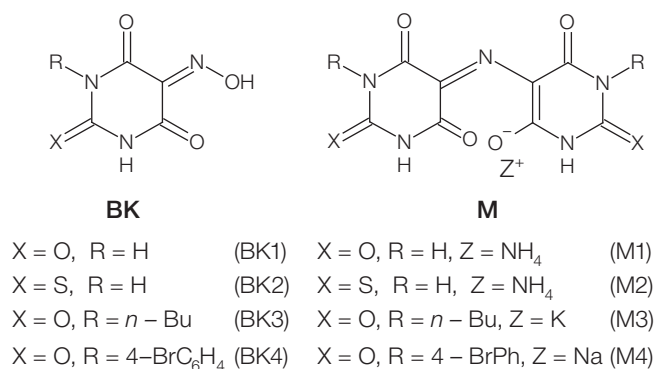


Figure prepared by the authors based on original data

Fig. 1. Structural formulas of violuric acids and salt derivatives of purpuric acid.

1-(4-bromophenyl)violuric acid (VA4) was officially registered as a hepatoprotective drug⁴.

This collectively positions violuric acids as a pharmacologically promising group, serving as a basis for the development of new drugs, particularly antihypoxants, actoprotectors, and antidotal agents, which are highly relevant for modern medicine. However, the biological targets and mechanisms of action of violuric acids remain unknown to date. Consequently, given the crucial role of drug metabolism data for screening and pharmaceutical development [15], investigating the patterns of distribution and biotransformation of violuric acids *in vivo* is of significant practical importance.

The aim of this study is to determine the structure of violuric acid metabolites and perform their quantitative assessment in an *in vivo* experiment.

MATERIALS AND METHODS

Physicochemical analysis

To confirm the molecular structure of the synthesized substances, nuclear magnetic resonance (NMR) spectroscopy was used. ¹H and ¹³C NMR spectra were recorded in DMSO-*d*₆ on a Bruker Avance 400WB spectrometer operating at 400 MHz and 100 MHz, respectively. Electronic spectra of the synthesized substances in the UV and visible regions were recorded on a Shimadzu UV-1800 scanning spectrophotometer using standard quartz cuvettes. Analysis of the purity of the synthesized substances, along with their identification and an assessment of their quantitative content in the biological media of experimental animals, was performed by high-performance liquid chromatography (HPLC) on a Shimadzu LC-20 Prominence chromatograph equipped with an autosampler and a diode array spectrophotometric detector. Separation was carried out on a Chromolith Performance-RP reversed-phase monolithic column having a length of 10 cm and at a

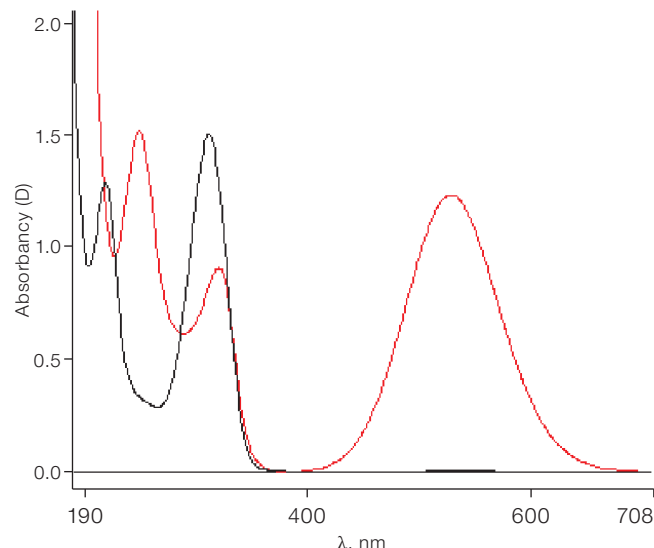


Figure prepared by the authors based on original data

Fig. 2. Absorption spectra of 1-butylvioluric acid (black) and its metabolite (red) at a concentration of 9×10^{-5} mol/L for each substance (water, pH 7.0)

column temperature of 40°C. The eluent flow rate was 5 mL/min, using a mobile phase of acetonitrile (phase A) and a 0.1% aqueous ammonium acetate solution (phase B) in a gradient elution mode. The gradient conditions were: A 1–30% (0–3 min), A 30% (3–5 min).

The analytical characteristics of the investigated substances and their metabolites, used for their identification and quantitative analysis in biological media, are presented in Table 1.

As an illustration, Figure 2 presents the electronic spectra of 1-butylvioluric acid (VA3) and its metabolite, *N,N'*-dibutylpurpurate (M3).

Synthesis of test and reference substances

The synthesis of all investigated substances was performed at the Golikov Scientific and Clinical Center of Toxicology. Violuric acids (VAs) were synthesized according to the general method described in patent [14]. The VA synthesis scheme consisted of two stages. In the first stage, the corresponding barbituric acid derivatives were obtained from substituted ureas and malonic ester. In the second stage, the target VAs were produced from these derivatives via nitrosation. The purity of the synthesized substances was not less than 98%. Purpuric acid in the form of its ammonium salt (M1, murexide) was a commercial product of AR (analytical reagent) grade (LenReaktiv, Russia). All other substances used in the work were commercially available products. The metabolites of violuric acid derivatives — 2,2'-dithiopurpuric acid (M2) and *N,N'*-dibutylpurpuric acid (M3) — were obtained in the form of ammonium and potassium salts,

⁴ State Register of Medicines. Moscow: Ministry of Health of Russia, Pharmaceutical Information Foundation; Marketing Authorization No. LP-00444 dated September 1, 2017 (In Russ.). https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=89b99d38-99b5-45b1-a65d-565936e3ded0

Table 1. Spectral and chromatographic characteristics of violuric and purpuric acids

Substance	λ_{max} , nm	E_{max} , $\text{M}^{-1} \times \text{cm}^{-1}$	RT, min
VA1	311.0	24,800	0.289
VA2	349.0	22,300	0.300
VA3	312.0	18,700	0.617
VA4	314.0	14,900	0.910
M1	521.0	10,400	0.300
M2	568.5	19,500	0.340
M3	528.5	12,900	1.832
M4	526.5	10,000	1.920

Table prepared by the authors based on original data

Note: λ_{max} — absorption maximum wavelength; E_{max} — molar extinction coefficient at the absorption maximum; RT — retention time.

respectively, according to the method described in reference [16], at a purity of not less than 95%.

Synthesis protocol for the sodium salt of N,N'-di-(4-bromophenyl)purpuric acid (M4). 0.005 mol (1.56 g) of 1-(4-bromophenyl)violuric acid (VA4) and 0.005 mol (1.41 g) of 1-(4-bromophenyl)barbituric acid [14] were dissolved in 10 mL of DMSO (dimethyl sulfoxide) at 50°C. 0.01 mol of ammonium acetate dissolved in 3 mL of methanol was added to this solution with stirring. The resulting mixture was stirred for 1 h at 50°C, then diluted with 25 mL of a 3% aqueous sodium bicarbonate solution and left for 1 h at room temperature for precipitate formation. The resulting precipitate was isolated using a Schott filter, washed with 10 mL of a 1% aqueous sodium bicarbonate solution, followed by 5 mL of distilled water, and then air-dried to constant weight. 1.35 g of a dark red powder of M4 with a decomposition temperature above 250°C was obtained. The yield of purpurate M4 was 78% of the theoretical at a purity of not less than 95%.

¹H NMR spectrum (DMSO-*d*₆), chemical shift (δ), multiplicity in parts per million (ppm): 7.23 (4H, Ar), 7.66 (4H, Ar), 10.5–11.5 broad singlet or br.s (2H, 2NH). ¹³C NMR spectrum (DMSO-*d*₆), δC, ppm: 121.1 (C⁵ + C^{5a}), 131.5 (4C_{Ar}), 131.6 (4C_{Ar}), 135.2 (4C_{Ar}), 149.9 (2C²O), 155–165 br.s (4CO).

The drug amtizole, used as a reference antihypoxant, was synthesized in the form of its succinate according to the method described in patent [17]; the purity of the preparation was not less than 98%.

The structure and purity of all synthesized substances were confirmed by NMR and HPLC methods.

Animals and housing

Experiments were conducted on outbred male white rats aged three months, weighing 200–240 g, procured from

the Rappolovo Laboratory Animal Nursery (Kurchatov Institute). The animals were housed under standard conditions with a 12-h light/dark cycle with ad libitum provision of standard rat chow and drinking water. Animals were randomized by weight when assigned to experimental groups.

Determination of violuric acids and their metabolites in rat urine

The study was conducted on 16 animals, which were divided into 4 groups of 4 individuals for each test substance. Administration was performed using an atraumatic gastric tube. Derivatives VA1 and VA2 were administered to animals at a dose of 80 mg/kg body weight as 5% aqueous suspensions with the addition of 2% Tween-80, while derivatives VA3 and VA4 were administered at a dose of 100 mg/kg body weight as 1% aqueous solutions with the addition of tris(oxyethyl) aminomethane (TRIS).

Following substance administration, the animals were placed in metabolic cages for urine collection, where they were housed for 24 h without access to food, while access to water was not restricted. Urine was collected at specified time intervals (4, 8 and 24 h) with volume recording. The obtained urine samples were stored in a freezer at –10°C for subsequent HPLC analysis. The methods for quantitative analysis of VAs and their metabolites in urine were developed and validated in accordance with domestic and international guidelines.⁵

For quantitative analysis, urine was diluted twofold with distilled water and analyzed by HPLC at an injection volume of 10 μL. Calibration solutions of the corresponding standards were chromatographed in parallel. A linear relationship between the chromatographic peak area and the concentration of the analyte was established in the range of 0.1–50 μg/mL. The quantitative content of

⁵ Beregovykh VV. Validation of analytical methods for drug manufacturers. Standard enterprise guideline for the production of medicinal products. Moscow; 2008 (In Russ.).

the compound in urine samples was calculated based on the chromatographic peak area and corresponding analytical characteristics as listed in Table 1.

Investigation of the metabolism of 1-butylvioluric acid (VA3) based on analysis of rat blood plasma and organs

The study was conducted on 20 animals, with 2 individuals per time point (15, 30, 60, 120, 180 min). To investigate the biotransformation and distribution of VA3 *in vivo*, the animals were divided into 2 groups of 10 individuals each, based on the route of administration: intraperitoneal (Group 1) and intragastric (Group 2). The dosage of VA3 in both cases was 50 mg/kg body weight; the test substance was administered to the animals as a 1% aqueous solution with the addition of 0.7% TRIS. Following the specified time intervals, the animals were euthanized by decapitation. After collecting whole blood (4–6 mL) from the rats, plasma was separated from erythrocytes by centrifuging the blood at 6000 rpm. Internal organs (liver and kidneys) were also collected. Plasma samples and organs were frozen immediately after collection and stored at -10°C for subsequent HPLC analysis.

Preparation of Blood Plasma Samples for HPLC Analysis. 0.5 mL of rat blood plasma was placed into a centrifuge tube with the addition of 1.0 mL of methanol, mixed, and the protein precipitate was separated by centrifugation for 5 min at 16,000 rpm. The supernatant was separated and analyzed by HPLC at an injection volume of 30 μL . The content of substances VA3 and M3 in the blood plasma samples was determined from the chromatographic peak area using the corresponding analytical characteristics provided in Table 1. The method for calculating the concentration of VA3 and its metabolite M3 in blood plasma was validated in accordance with domestic and international guidelines.⁶

Preparation of liver tissue samples for HPLC analysis. 0.5 g of rat liver sample was ground in an agate mortar to produce a homogeneous paste. The ground material was mixed with 1 mL of methanol, transferred to a centrifuge tube, and centrifuged for 5 min at 16,000 rpm. The supernatant was separated and analyzed by HPLC, with an injection volume of 30 μL . Preparation of kidney tissue samples for HPLC analysis was performed in the same manner as for the liver.

The content of substance VA3 and its metabolite M3 in the liver and kidney samples was determined from the chromatographic peak area using the corresponding analytical characteristics provided in Table 1.

To study the anti-hypoxic activity of 1-butylvioluric acid (VA3) and its metabolite, potassium *N,N'*-dibutylpurpurate (M3), four experimental groups ($n = 6$) were formed: No. 1 — control; No. 2 — reference

standard; Nos. 3 and 4 — test groups. The study was conducted using a model of hemic hypoxia induced by a lethal dose of sodium nitrite, in accordance with methodological guidelines for the investigation of anti-hypoxic agents.⁷ The protective activity was assessed under conditions of prophylactic intraperitoneal administration of the substances. The drug amtirole, recognized as one of the most universal antihypoxants [18], was used as the reference substance.

Control animals in group No. 1 were administered 1 mL of a 0.9% sodium chloride solution. Animals in group No. 2 were administered amtirole succinate at a dose of 100 mg/kg as a 10% aqueous solution. Animals in groups No. 3 and No. 4 were administered with the test substances VA3 and M3, respectively, as 7.5% aqueous solutions, at substance dosages of 75 mg/kg.

Sodium nitrite (AR grade, LenReaktiv, Russia) was administered to rats subcutaneously as a 2% aqueous solution 30 min after the administration of the test substances. The dose of sodium nitrite was 100 mg/kg, which is 25% higher than the lethal dose for rats ($\text{LD}_{100} = 80 \text{ mg/kg}$). During the experiment, the animals were housed in enclosures of 6 individuals each for free observation of the clinical picture.

The clinical manifestations of the action of sodium nitrite and the test substances were recorded during the experiment. Animal death was recorded at the moment of cardiac arrest. Observations of surviving animals were conducted for 3 days. The criteria for the efficacy of the test substances were the increase in animal survival time and the survival percentage compared to the control. Statistical processing (calculation of mean value and absolute error) of the data was performed using Microsoft Office Excel software.

RESULTS AND DISCUSSION

The design of the *in vivo* study was primarily aimed at identifying the qualitative composition of violuric acid metabolites while minimizing the number of animals used in the experiments.

The determination of metabolites in animal urine was conducted following intragastric administration of the corresponding substances VA1–VA4. The urine collection time points were chosen to assess the initial, intermediate, and final stages of excretion. When analyzing the urine composition of the experimental animals by HPLC, corresponding derivatives of purpuric acid M1–M4 were detected in all cases, alongside the unchanged compounds VA1–VA4. The corresponding structures are presented in Figure 1.

Identification of purpurates in urine was performed in each case by comparing the retention time on the chromatogram and the diode-array spectrum with synthesized reference substances M1–M4. As noted earlier,

⁶ Ibid

⁷ Lukyanova LD, ed. Methodological recommendations for the experimental study of drugs proposed for clinical investigation as anti-hypoxic agents. Moscow: USSR Ministry of Health, 1990.

purpurates possess characteristic absorption spectra in the UV and visible regions (Table 1 and Fig. 2), which allowed for their unambiguous identification in biological media using the synthesized standards M1–M4.

Quantitative indicators of the excretion of violuric acids and their metabolites in rat urine over the corresponding time intervals are presented in Table 2 and Figure 3.

The obtained results indicate that the formation of purpurates is a general pattern of biotransformation for violuric acids in the rat organism. Among the substances considered, 1-butylvioluric acid (VA3) undergoes the most extensive metabolism, with 47% excreted in urine as *N,N'*-dibutylpurpurate (M3) and 53% excreted unchanged. The substrate least prone to metabolism is 1-(4-bromophenyl)violuric acid (VA4), for which the conversion rate to its metabolite is only 15%.

The observed metabolic transformation of violuric acids is quite unusual and can even be considered unique. Judging by the structure of the formed purpurates, the biotransformation of violuric acids involves the condensation and cross-linking of two fragments of the original molecule. This cannot be classified among the well-known pathways of xenobiotic metabolism, such as oxidation, hydrolysis, conjugation, and other common transformations [19]. Since, given the low substrate concentration in the organism, the random cross-linking of two VA3 molecules seems unlikely, this transformation apparently requires a specific biochemical mechanism. This is particularly relevant since such a reaction is not characteristic for violuric acids in *in vitro* solutions, even at significantly higher concentrations.

To explain the ongoing process, we propose a mechanism schematically depicted in Figure 4. From a chemical standpoint, it is evident that one of the reaction stages involves the reduction of the oxime group in VA3. However, it is more challenging to explain how the reduced intermediate couples with the original molecule under physiological conditions. Here, the complexing properties known to be possessed by violurates [20] may play a key role. As a result of complex 1 formation with a multivalent metal cation (e.g., iron, zinc, calcium, or another cation), two VA molecules are grouped around a single coordinating center. Subsequent enzymatic reduction of the oxyimino group, forming intermediate 2, and its condensation via a nucleophilic substitution mechanism appears to be a logical continuation of the process, leading to the formation of a new chelating system — the purpurate molecule M.

According to preliminary data,⁸ substance VA3 was of the greatest interest among the VA1–VA4 derivatives in terms of its anti-hypoxic activity and was therefore selected for in-depth investigation. In the course of the present work, we assessed the concentration kinetics of 1-butylvioluric acid (VA3) and its metabolite (M3) in rat blood plasma following intragastric and intraperitoneal administration of VA3. The results are presented in Table 3.

Metabolite M3 appeared in the blood plasma as early as 15 min at a concentration of 0.10 mg/L after intragastric- and 0.38 mg/L after intraperitoneal administration of 1-butylvioluric acid, indicating rapid metabolism (Fig. 5). Furthermore, judging by the fact that the maximum

Table 2. Average amounts of parent substances and their metabolites excreted in urine following intragastric administration of violuric acids

Parent substance (dose, mg/kg)	Products in urine	Excreted in urine, mg/kg			Total excreted over 24 h	
		Time period, h			mg/kg	% of administered dose
		0–4	4–8	8–24		
VA1 (80)	VA1	4.27	4.37	0.30	8.94	11.2 ± 1.8
	M1	1.57	1.76	0.37	3.70	4.63 ± 0.91
VA2 (80)	VA2	1.25	0.30	0.28	1.83	2.29 ± 0.35
	M2	0.27	0.071	0.023	0.36	0.45 ± 0.12
VA3 (100)	VA3	8.35	3.24	1.83	13.42	13.42 ± 2.26
	M3	4.94	4.99	2.32	12.25	12.25 ± 1.92
VA4 (100)	VA4	5.12	0.91	0.18	6.21	6.21 ± 1.45
	M4	0.95	0.12	0.012	1.08	1.08 ± 0.34

Table prepared by the authors based on original data

⁸ Search for anti-hypoxants and actoprotectors in the series of violuric acid derivatives: research report / Head: Krasnov KA; Executors: Krasnova AA, Lapina NV, Ivnitsky YuYu, et al.; Golikov Scientific and Clinical Center of Toxicology. St. Petersburg, 2024. Reg. No. SRW 124022400178-1.

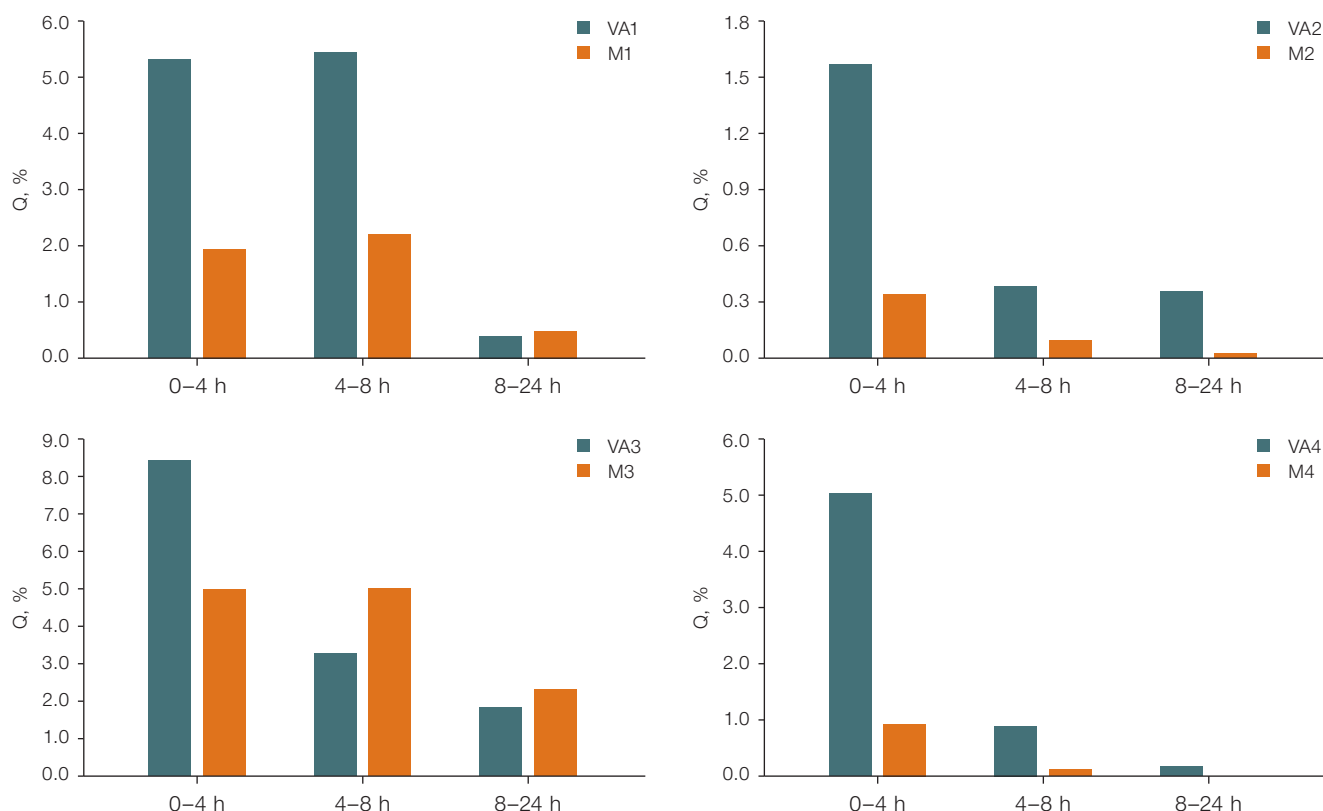


Figure prepared by the authors based on original data

Fig. 3. Excretion kinetics of violuric acids and their metabolites in rat urine following intragastric administration: data are presented as mean values; $Q\%$ — mass amount of excreted substance as a percentage of the administered dose

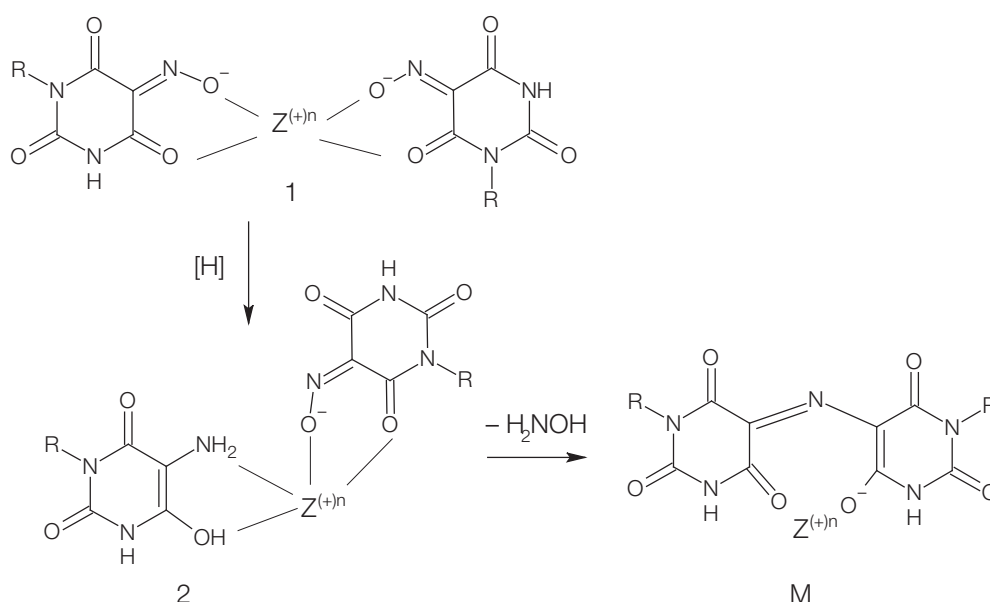


Figure prepared by the authors based on original data

Fig. 4. Hypothetical mechanism of violuric acid transformation into purpurate *in vivo*

concentration of M3 did not exceed 0.75 mg/L, no significant accumulation of the metabolite in the blood was observed, which was associated with its faster elimination via the kidneys into urine compared to the parent

substance. The purpurate was also detected in the livers and kidneys of rats. The maximum concentration after intraperitoneal administration was reached after 1 h, i.e., later than in the blood. The maximum purpurate

Table 3. Concentration of substances in blood plasma following intragastric and intraperitoneal administration of 1-butylvioluric acid

Time, min	Substance	Concentration, mg/L	
		Intragastric administration	Intraperitoneal administration
15	VA3	16.6 ± 2.5	70.8 ± 19.5
	M3	0.10 ± 0.05	0.38 ± 0.40
30	VA3	27.4 ± 3.9	29.3 ± 5.3
	M3	0.13 ± 0.03	0.74 ± 0.24
60	VA3	16.9 ± 2.7	8.7 ± 2.3
	M3	0.43 ± 0.11	0.75 ± 0.19
120	VA3	5.5 ± 1.2	1.9 ± 0.5
	M3	0.35 ± 0.14	< 0.05
180	VA3	3.9 ± 1.2	1.8 ± 0.5
	M3	0.09 ± 0.02	< 0.005

Table prepared by the authors based on original data

Note: VA3 — 1-Butylvioluric acid; M3 — *N,N'*-Dibutylpurpuric acid.

content in the liver homogenate was 0.46 mg/kg, which is 1.6 times lower than in the blood, while in the kidneys it was 1.23 mg/kg, which is 1.6 times higher than in the blood.

To address the question of the biological activity of metabolite M3, we conducted an experimental study, which demonstrated that this product possesses pronounced anti-hypoxic activity in the hemic hypoxia model in rats. Prophylactic administration of potassium *N,N'*-dibutylpurpurate (M3) completely prevented the death of animals that received a lethal dose of sodium nitrite. A similar protective effect was demonstrated by 1-butylvioluric acid (VA3) (Table 4).

The clinical picture of poisoning in rats developed 25–30 min after the subcutaneous administration of nitrite. Ataxia and dyspnea, followed by convulsions and

death of the animals were observed in the control group. In rats pre-treated with substances VA3 and M3, symptoms of poisoning were also noted; however, these were less pronounced and practically disappeared 2 h following nitrite injection. Starting from the 4th h, all animals in these groups appeared completely healthy. Under the same conditions, the reference drug amtizole did not prevent animal death, only prolonging their survival time by 23%.

Thus, it is established that M3 is an active metabolite of acid VA3 and likely plays a role in the development of the latter's pharmacological effect. This result also indicates that purpurates, whose biological activity has not been previously investigated, may be of interest as potential agents for the treatment of poisoning by methemoglobin-forming agents.

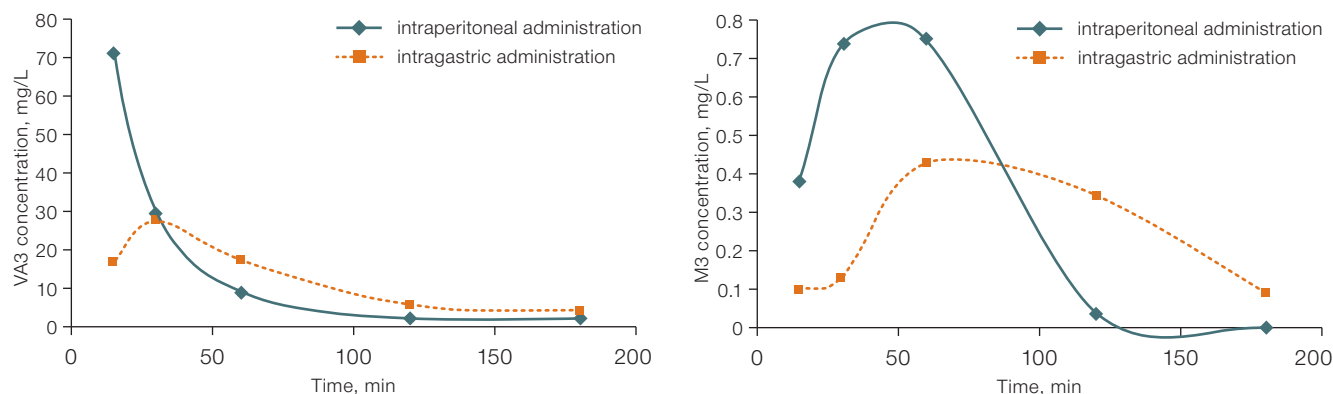


Figure prepared by the authors based on original data

Fig. 5. Concentration kinetics of 1-butylvioluric acid (left) and its metabolite (right) in rat blood plasma following its intraperitoneal and intragastric administration

Table 4. Protective efficacy of substances in rats under conditions of lethal sodium nitrite poisoning

Substance	Dose, mg/kg	Survival rate, %	Mean survival time	
			min (± ΔX)	% of control
Control (water)	–	0	46.1 (± 4.1)	100
Amtizole succinate	100	0	56.8 (± 4.5)	123
VA3	75	100	No mortality	
M3	75	100	No mortality	

Table prepared by the authors based on original data

Note: ΔX — absolute error of values; «—» — administered 1 mL of water without the active substance.

CONCLUSION

- 1. Violuric acids are metabolized in animals’ organisms, yielding corresponding derivatives of purpuric acid.
- 2. The biotransformation mechanism of violuric acids into purpurates involves reduction of the oximino group and cross-condensation with a molecule of the original substrate.

- 3. Violuric acids and their metabolites are largely excreted from the body via the kidneys in urine.
- 4. 1-butylvioluric acid (VK3) and its metabolite N,N'-dibutylpurpuric acid (M3) exhibit pronounced protective activity against sodium nitrite poisoning.
- 5. Purpuric acids, along with violuric acids, are of interest for further study as potential agents for controlling poisoning with methemoglobin-forming toxins.

References

1. Starokon PM, Khanevich MD. Antihypoxants in surgery: development prospects. *Hospital Medicine: Science and Practice*. 2022;5(3):56–62 (In Russ.).
EDN: [GVBQTE](#)

2. Oganov RG. Positive experience of ethylmethylhydroxypyridine succinate usage in cardiological patience. *Cardiovascular Therapy and Prevention*. 2017;16(5):91–4 (In Russ.).
<https://doi.org/10.15829/1728-8800-2017-5-91-94>

3. Golovko AI, Batocyrenova EG, Komov JuV, Khalchitsky SE, Kashuro VA. Review of drugs for the correction of CND disorders developed as a result of the action of neurotoxicants. *Medline.ru*. 2022;23:385–419 (In Russ.).
EDN: [WEDJBD](#)

4. Shustov EB, Karkishhenko VN, Semjonov HH, Okovity SV, Bolotova VTs, Yuskovets VN. Search of regularities, determining antihypoxic activity of the compounds with nootropic and neurotropic action. *Journal Biomed*. 2015;1:18–23 (In Russ.).
<https://doi.org/10.13140/RG.2.1.2037.3200>

5. Oliynyk S, Oh S. The pharmacology of actoprotectors: practical application for improvement of mental and physical performance. *Biomolecules and Therapeutics*. 2012;20(5):446–56.
<https://doi.org/10.4062/biomolther.2012.20.5.446>

6. Shamrey VK, Kurasov ES, Nechiporenko VV, Kolchev AI, Tsygan NV. Possibilities of using Mexidol in the complex therapy of mental disorders. *S.S. Korsakov Journal of Neurology and Psychiatry*. 2020;120(5):160–4 (In Russ.).
<https://doi.org/10.17116/jnevro2020120051160>

7. Shabanov PD, Zarubina IV. Hypoxia and antihypoxants, focus on brain injury. *Reviews on Clinical Pharmacology and Drug Therapy*. 2019;17(1):7–16 (In Russ.).
<https://doi.org/10.17816/RCF1717-16>

8. Fedorov VN, Petrovsky AK, Vdovichenko VP, Zakharova MN, Arshinov AV. Problems of classification and characteristics of neurotropic agents used for the treatment of cerebrovascular accidents. *Medical Ethics*. 2022;1:25–33 (In Russ.).
EDN: [JYWWBB](#)

9. Repina EF, Khusnutdinova NU, Timasheva GV, Baygildin SS, Karimov DO, Musina LA, et al. Morphological specificities of hepatoprotective effects of antihypoxants in experimental acute liver damage with carbon tetrachloride. *Toxicological Review*. 2019;1(154):43–8 (In Russ.).
<https://doi.org/10.36946/0869-7922-2019-1-43-48>

10. Okovityj SV, Suhanov DS, Zaplutanov VA, Smagina AN. Antihypoxants in current clinical practice. *Clinical Medicine (Russian Journal)*. 2012;90(9):63–8 (In Russ.).
EDN: [PUHHAZ](#)

11. Urakova NA, Urakov AA. New-generation antihypoxants: Alkaline hydrogen peroxide solutions as generators of medical oxygen gas. *Psychopharmacology and Biological Narcology*. 2025;16(1):35–42 (In Russ.).
<https://doi.org/10.17816/phbn642337>

12. Krasnov KA, Krasnova AA, Feklistova KA, Papp VT. Violuric acids: history, pharmacology and prospects (analytical review). *Medline.ru*. 2024;25:630–47 (In Russ.).
EDN: [ALHSLT](#)

13. Shugalej IV, Celinskij IV, Krasnov KA, Sedel'nikova NA. The inhibitory effect of violuric acid derivatives in the oxidation reaction of oxyhemoglobin with nitrite ion. *Russian Journal of General Chemistry*. 1993;63(7):1646–50 (In Russ.).

14. Ashkinazi RI. *N-Substituted derivatives of 5-hydroxyimino-barbituric acid*. Patent of the Russian Federation No. 2188196;2002 (In Russ.).
EDN: [ADPSVJ](#)

15. Liu L, Liu Y, Zhou X, Xu Zh, Zhang Y, Ji L, et al. Analyzing the metabolic fate of oral administration drugs: A review and state-of-the-art roadmap. *Pharmacology*. 2022;13:962718.
<https://doi.org/10.3389/fphar.2022.962718>

16. Krasnov KA, Feklistova KA, Krasnova AA, Papp VT, Gaft SS. Synthesis and Properties of N-Substituted Purpuric Acid Derivatives and Their 2-Thioanalogues. *Journal of General Chemistry*. 2024;94(9):958–64 (In Russ.).
EDN: [ROZXFJ](#)

17. Konev VF, Tomchin AB, Vinogradov VM, Maslenikov AI, Kostycheva MV, Zjukina GV, et al. *3,5-Diamino-1,2,4-thiadiazole succinate showing antihypoxic activity*. Patent of the USSR No. 1584340;1996 (In Russ.). EDN: [EXYAFC](#)
18. Marysheva VV. Antihypoxants of the aminothiols series. *Reviews on Clinical Pharmacology and Drug Therapy*. 2007;5(1):17–27 (In Russ.). EDN: [HZLMGN](#)
19. Kutsenko SA. *Fundamentals of Toxicology*. St. Petersburg: Foliant; 2004 (In Russ.). EDN: [QKMWIB](#)
20. Lorenz V, Liebing P, Engelhardt F, Stein F, Kuhling M, Schroder L, et al. Review: the multicolored coordination chemistry of violurate anions. *Journal of Coordination Chemistry*. 2019;72(1):1–34. <https://doi.org/10.1080/00958972.2018.1560431>

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EXPERIMENTAL VALIDATION OF APPROACHES TO PANCURONIUM BROMIDE USE FOR CORRECTION OF MYASTHENIC SYNDROME IN ANTICHOLINESTERASE POISONING

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Introduction. The problem of intermediate syndrome following anticholinesterase poisoning remains poorly studied. This syndrome is clinically manifested as a myasthenic condition developing after the cholinergic crisis, affecting the muscles of the face, neck, proximal limbs, and respiratory muscles. The current literature describes limited attempts to use non-depolarizing muscle relaxants for the prevention and treatment of this condition. However, given the diversity of mechanisms underlying toxic myasthenia in anticholinesterase poisoning and the low safety profile of non-depolarizing muscle relaxants, research into the effective and toxic doses of these drugs is highly relevant for establishing the principles of their use in correcting neuromuscular transmission disorders.

Objective. To experimentally evaluate the efficacy of pancuronium bromide, a non-depolarizing muscle relaxant, in correcting neuromuscular transmission impairments in cases of anticholinesterase poisoning.

Materials and methods. Experimental, two-stage studies were conducted using male outbred white rats weighing 220–250 g ($n = 78$). Initially, pancuronium bromide was administered subcutaneously to intact animals to determine effective doses. The severity and duration of the muscle relaxant effect were assessed using clinical and functional tests (hanging on a horizontal bar, assessment of movement impairments according to the De Bleeker scale), and electromyography (single and rhythmic stimulation at 30 Hz). Subsequently, therapeutic doses of the drug were determined in a rat model of fenthion poisoning (12 h after its single subcutaneous administration at an LD_{50} dose of 479.4 mg/kg) based on data from the aforementioned methods and evaluation of changes in animal mortality rates. Statistical analysis of the results was performed using non-parametric statistical methods in the Prism GraphPad 9.0 software environment.

Results. Based on the assessment of neurological status and electromyography results, the median effective dose (ED_{50}) of pancuronium upon subcutaneous administration in intact rats was found to be 238.0 [95% CI: 219.8; 257.7] $\mu\text{g/kg}$. In contrast, against the background of severe fenthion poisoning, its therapeutic dose was statistically significantly lower ($p < 0.05$, Student's t -test), amounting to 90.1 [95% CI: 77.3; 105.1] $\mu\text{g/kg}$. Similar trends were observed for the median lethal doses (LD_{50}) of pancuronium bromide: 321.1 [95% CI: 305.8; 337.1] $\mu\text{g/kg}$ and 152.3 [130.6; 177.6] $\mu\text{g/kg}$, respectively. Administration of pancuronium at the median therapeutic dose reduced the severity of the myasthenic syndrome induced by fenthion poisoning, manifesting as restored muscle strength and normalized electrophysiological characteristics of neuromuscular transmission.

Conclusions. The experiment demonstrated that pancuronium bromide can be used to correct neuromuscular transmission disorders underlying the intermediate syndrome emerging as a result of anticholinesterase poisoning. The effective dose for this correction is 90.1 $\mu\text{g/kg}$, which is 2.6-fold lower than the effective dose for healthy animals (238.0 $\mu\text{g/kg}$). The main electrophysiological criteria for the regression of the neuromuscular block should include a reduction in the number of repeated M-responses and the restoration of the decrement-increment pattern of the M-response series, which persist for 1 h after pancuronium bromide administration.

Keywords: pancuronium bromide; organophosphorus compound; intermediate syndrome; electroneuromyography; clinical-functional tests; toxic myasthenia

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ЭКСПЕРИМЕНТАЛЬНОЕ ОБОСНОВАНИЕ ПОДХОДОВ К ПРИМЕНЕНИЮ ПАНКУРОНИЯ БРОМИДА ДЛЯ КОРРЕКЦИИ МИАСТЕНИЧЕСКОГО СИНДРОМА ПРИ ОТРАВЛЕНИЯХ АНТИХОЛИНЭСТЕРАЗНЫМИ ЯДАМИМ.А. Тюнин, Н.С. Ильинский[✉], С.В. Чепур, В.А. Мацейчик, Е.Ю. Ижорская

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Введение. До настоящего времени малоизученной остается проблема промежуточного синдрома при отравлениях антихолинэстеразными веществами, который клинически представляет собой развивающийся после холинэргического криза миастенический синдром, затрагивающий мышцы лица, шеи, проксимальных отделов конечностей и дыхательные мышцы. В литературе описаны немногочисленные попытки применения недеполяризующих миорелаксантов с целью профилактики и лечения промежуточного синдрома. Вместе с тем, учитывая многообразие механизмов токсической миастении при отравлениях антихолинэстеразными ядами и низкую безопасность недеполяризующих миорелаксантов, представляются актуальными исследования по определению эффективных и токсических доз рассматриваемых лекарственных средств в целях обоснования принципов их применения для коррекции нарушений нервно-мышечной передачи.

Цель. Экспериментально оценить эффективность недеполяризующего миорелаксанта панкурония бромида в отношении коррекции нарушений нервно-мышечной передачи при отравлениях антихолинэстеразными ядами.

Материалы и методы. Экспериментальные исследования проведены на самцах белых беспородных крыс массой 220–250 г ($n = 78$) в два этапа. Первоначально недеполяризующий миорелаксант панкурония бромид вводили подкожно интактным животным для определения его эффективных доз по развитию миорелаксации. Выраженность и длительность миорелаксанта эффекта оценивали при помощи клинко-функциональных тестов (подтягивание на горизонтальной перекладине, нарушения движений по шкале J. De Bleecker) и электромиографии (одиночная и ритмическая стимуляция с частотой 30 Гц). Затем определяли терапевтические дозы препарата на модели отравления крыс фентионом (через 12 ч после однократного подкожного введения в дозе $LD_{50} = 479,4$ мг/кг) по данным указанных методов исследований и оценки изменения частоты гибели животных. Статистический анализ результатов проводили методами непараметрической статистики при помощи программного обеспечения Prism GraphPad 9.0.

Результаты. На основании данных оценки неврологического статуса и результатов электромиографии было установлено, что значение средней эффективной дозы панкурония при подкожном введении у интактных крыс составило 238,0 [95% ДИ: 219,8; 257,7] мкг/кг, в то время как на фоне тяжелого отравления фентионом значение его терапевтической дозы статистически значимо ниже ($p < 0,05$, t -критерий Стьюдента) и равно 90,1 [95% ДИ: 77,3; 105,1] мкг/кг. Аналогичные закономерности прослежены и для средних смертельных доз панкурония бромида: 321,1 [95% ДИ: 305,8; 337,1] и 152,3 [130,6; 177,6] мкг/кг соответственно. Введение панкурония в средней терапевтической дозе снижало выраженность миастенического синдрома, вызванного отравлением фентионом, в виде восстановления мышечной силы и нормализации электрофизиологических характеристик нервно-мышечной передачи.

Выводы. Экспериментально показано, что с целью коррекции расстройств нервно-мышечной передачи, лежащих в основе промежуточного синдрома при отравлениях антихолинэстеразными ядами, может быть использован панкурония бромид в сниженной в 2,6 раза (90,1 мкг/кг) относительно эффективной для здоровых животных (238,0 мкг/кг) дозе. К основным электрофизиологическим критериям регресса нервно-мышечного блока следует относить сокращение числа повторных М-ответов и восстановление декремент-инкремента серии М-ответов, сохраняющиеся в течение 1 ч после введения панкурония бромида.

Ключевые слова: панкурония бромид; фосфорорганическое соединение; промежуточный синдром; электромиография; клинко-функциональные тесты; токсическая миастения

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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 «ГНИИИ ВМ» МО РФ (протокол № 9 от 27.04.2020).

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INTRODUCTION

For decades, research conducted by the Russian school of toxicology has been focused on the development of approaches to preventing and treating cases of anticholinesterase poisoning. These efforts have resulted in the appearance of effective antidotes and agents for pathogenetic therapy [1, 2]. However, these antidotal drugs fail

to counteract the entire spectrum of toxic effects produced by anticholinesterase compounds (AChEs), which are associated with severe delayed effects (intermediate syndrome, delayed polyneuropathies) [1–3].

To date, the intermediate syndrome (IS) emerging during poisoning with AChEs remains poorly studied [4]. Clinically, IS presents as a myasthenic syndrome developing after the cholinergic crisis, affecting the muscles of

the face, neck, proximal limbs, and respiratory muscles [5]. According to clinical studies, IS develops 24–98 h after the poison enters the body. The duration of IS varies from 5 to 30 days. The most severe IS manifestation involves the paralysis of the respiratory muscles, leading to progressive respiratory failure. The incidence of IS ranges 10.0–68.0%, depending on the type of toxicant, with the mortality rate reaching 25.0–40.0% [7].

The Russian toxicology school regards IS as Phase II of toxic myopathy or as Type 2 paralysis [8]. Toxic myopathy is understood as a unified pathological process at the level of the neuromuscular synapse, characterized by the development of a neuromuscular block (NMB). Clinically, the course of toxic myopathy is conventionally divided into two phases. Phase I involves manifestations of a spastic neuromuscular block (Type 1), presenting as widespread myofibrillations and muscle rigidity. Phase II corresponds to the onset of a paralytic NMB, manifesting as progressive weakness of the skeletal musculature [5, 8, 9].

Although the neuromuscular transmission impairments underlying IS have been studied [3, 5], a unified consensus on its pathogenesis and treatment has yet to be established. In particular, no effective methods for treating IS have been developed to date. It is believed that the main therapeutic measures are based on conventional approaches for etiotropic and detoxification therapy recommended for AChE poisonings. The cornerstone of IS management remains the timely detection of respiratory muscle paralysis; upon its onset, immediate initiation of artificial ventilation (AV) with prophylactic administration of antibacterial agents is indicated [6, 9].

Given the key role of NMB in the pathogenesis of IS [10], a limited number of studies have reported data on the successful use of non-depolarizing muscle relaxants (NDMRs) in the integrated therapy of AChEs poisoning [11]. Experimental and clinical studies have demonstrated the positive effects of rocuronium and atracurium [11, 12], leading the authors to recommend further investigation into the efficacy and safety of NDMRs. Therefore, the development of dosing strategies and optimal administration protocols for their use in AChE poisonings is a relevant research direction [3, 4].

In this work, we experimentally evaluate the efficacy of pancuronium bromide, a non-depolarizing muscle relaxant, in correcting neuromuscular transmission impairments in cases of anticholinesterase poisoning.

MATERIALS AND METHODS

The study was performed using male outbred white rats weighing 220–250 g (Rappolovo Breeding Nursery, Leningrad Oblast). The animals were housed in a vivarium under standard zoohygienic conditions and regulations. The experimental procedures were conducted in accordance with the requirements of the Order of the Ministry of Health of Russia.¹

The study was conducted in two stages. During the first stage, the duration of muscle relaxation, effective doses, and toxic doses of the long-acting aminosteroid non-depolarizing muscle relaxant (NDMR) pancuronium bromide (Sigma-Aldrich, USA) were determined in intact rats following a single subcutaneous (s.c.) administration at doses ranging 200–350 µg/kg. Using a block randomization method, seven experimental groups were formed, each consisting of six rats: Group 1 — 200 µg/kg; Group 2 — 225 µg/kg; Group 3 — 250 µg/kg; Group 4 — 280 µg/kg; Group 5 — 300 µg/kg; Group 6 — 320 µg/kg; Group 7 — 350 µg/kg.

Electroneuromyography (ENMG) was performed using a Neuro-MVP-4 electromyograph (Neurosoft, Russia) and the Neuro-MVP-Omega software in accordance with a previously described methodology without the use of anesthetics [13]. To record the muscle relaxant effect of pancuronium bromide, ENMG was conducted in rhythmic stimulation (RS) mode at a frequency of 30 Hz, since high-frequency RS has been established as the most informative test for diagnosing neuromuscular transmission (NMT) impairments. The electrophysiological criteria for achieving the target effect of the muscle relaxant were defined as minimally evident signs of NMT block on ENMG, specifically a 10.0% decrease in the M-response amplitude (area) from baseline values upon single stimulation and/or the appearance of a decrement in amplitude (area) exceeding 10.0% during rhythmic stimulation. Neurological deficit and ENMG were monitored at two-minute intervals for 2 h after administration of the muscle relaxant.

During the second stage of the study, using the aforementioned diagnostic methods, the effect of pancuronium bromide administered subcutaneously across a dose range on the dynamics of NMT impairments in a toxic rat model was evaluated. This evaluation was conducted 12 h after the application of fenthion (IUPAC: dimethoxy-(3-methyl-4-methylsulfanyphenoxy)-sulfanylidene-λ5-phosphane, Adamanth, Russia) at the median lethal dose ($LD_{50}/1 \text{ day} = 479.4 \text{ mg/kg bw}$ subcutaneously). The selection of fenthion as a model toxicant was based on the high frequency of IS manifestation in poisoning events [5, 7]. Using the block randomization method, six experimental groups were formed, each consisting of six rats: Group 1 — fenthion 479.4 mg/kg + pancuronium bromide 64.1 µg/kg; Group 2 — fenthion 479.4 mg/kg + pancuronium bromide 83.3 µg/kg; Group 3 — fenthion 479.4 mg/kg + pancuronium bromide 108.3 µg/kg; Group 4 — fenthion 479.4 mg/kg + pancuronium bromide 140.8 µg/kg; Group 5 — fenthion 479.4 mg/kg + pancuronium bromide 183.0 µg/kg; Group 6 — fenthion 479.4 mg/kg + pancuronium bromide 238.0 µg/kg.

Statistical analysis of the data was performed using the Prism GraphPad 9.0 software (GraphPad Software, USA) in the Windows 10 operating system. Multiple comparisons of unpaired samples were conducted using the Kruskal–Wallis test. Comparison of binary qualitative

¹ Order of the Ministry of Health of the Russian Federation № 199n «On Approval of the Rules of Good Laboratory Practice» dated 01.04.2016.

Table 1. Results of the 30 Hz rhythmic stimulation test in rats after single subcutaneous administration of pancuronium bromide

Dose, µg/kg bw	Decrement incidence, %	Decrement value, %	Onset time of effect, min	Duration of effect, min	Mortality rate, %
200	0.0	4.5 [2.1; 6.3]	–	–	0.0
225	33.3	10.5 [8.4; 12.5]	8.0 [8.5; 9.5]	42.0 [41.0; 43.0]	0.0
250	66.7	26.0 [15.5; 36.4]	8.0 [7.5; 8.5]	41.0 [40.0; 42.5]	0.0
280	83.3	68.1 [48.6; 83.1]	6.0 [4.0; 6.0]	60.0 [58.0; 62.0]	0.0
300	100.0	79.7 [58.5; 87.9]	4.0 [2.5; 4.0]	70.0 [64.0; 74.0]	16.7
320	100.0	97.3 [96.0; 98.7]	2.0 [2.0; 3.5]	66.0 [62.0; 68.0]	50.0
350	100.0	98.2 [96.5; 99.5]	2.0 [2.0; 2.0]	8.5 [6.0; 9.5]	100.0

Table compiled by the authors based on original data

Note: quantitative values of the recorded parameters are presented as the median, first, and third quartiles — $M_e [Q_1; Q_3]$.

data was performed using Fisher’s exact test. Effective and lethal doses were calculated using Finney’s probit analysis in the Statistics 2005+ software (Russia). Doses are presented as the mean value and 95% confidence interval. Differences between groups were considered statistically significant at $p < 0.05$.

RESULTS

Determination of effective and toxic doses of pancuronium bromide in intact rats

During the first stage of the study, the effective and toxic doses of pancuronium bromide administered to intact animals were determined. Based on the assessment of the neurological status of rats using

CFTs, a single subcutaneous administration of pancuronium bromide in the dose range of 200–225 µg/kg led to the development of myasthenic syndrome in 33.3–66.7% of cases, while administration of doses ranging 250–350 µg/kg resulted in its manifestation in 100% of cases. The speed of onset and severity of the myasthenic syndrome in intact rats exhibited a dose-dependent relationship. On average, the initial signs of the muscle relaxant effect were observed 5–7 min after administration, manifesting as reduced spontaneous motor activity, bilateral ptosis, and the inability to perform or maintain the position during the horizontal bar hanging test. The severity of paralysis then progressed: postural changes were detected, including loss of support on the distal limbs, inability to hold the head up, ataxia, and adynamia.

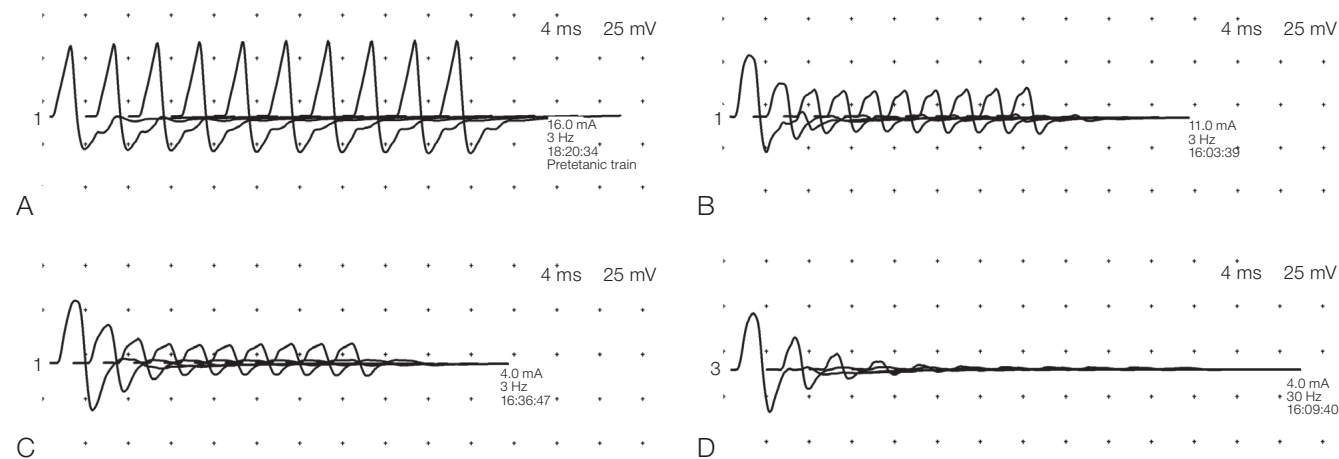


Figure prepared by the authors

Fig. 1. Typical M-response series of the gastrocnemius muscles in intact rats during the rhythmic stimulation test following a single subcutaneous administration of pancuronium bromide: A — no decrement observed during baseline assessment; B — 15 min after administration of a 280 µg/kg bw dose (decrement 50.6%); C — 20 min after administration of a 280 µg/kg bw dose (decrement 79.9%); D — 10 min after administration of a 300 µg/kg bw dose (decrement 96.7%); X-axis: M-response duration, ms; Y-axis: M-response amplitude, mV

Administration of pancuronium bromide at doses of 300–350 µg/kg bw resulted in animal death due to the neuromuscular form of acute respiratory failure within 5–10 min. The mortality rate in each group is presented in Table 1.

The dynamics of the NMB induced by the administration of various pancuronium doses were recorded using ENMG in rhythmic stimulation mode (Table 1). Figure 1 clearly illustrates characteristic changes in the M-response series, showing decrements in amplitude and area depending on the dose and time after muscle relaxant administration. The criteria for the NMB effect were defined as external signs of peripheral paresis combined with the illustrated electrophysiological changes.

The obtained data allowed us to establish that the median effective dose (ED_{50}) of pancuronium bromide following a single subcutaneous administration to intact rats was 238.0 [219.8; 257.7] µg/kg (ED_{16} = 211.4 µg/kg, ED_{84} = 274.4 µg/kg). The median duration of muscle relaxation was 60.0 [44.7; 64.0] min. Furthermore, the median lethal dose (LD_{50}) of pancuronium bromide following a single subcutaneous administration to intact animals was 321.1 [305.8; 337.1] µg/kg (LD_{16} = 298.1 µg/kg, LD_{84} = 339.1 µg/kg), and the median time to death was 26.0 [24.5; 27.5] min.

Thus, the therapeutic index, calculated as the ratio of LD_{50}/ED_{50} , for subcutaneously administered pancuronium bromide was 1.35. The ratio of LD_1 (255.0 µg/kg) to ED_{99} (292.0 µg/kg), referred to as the certain safety factor (CSF), equaled 0.87. These parameters indicate a narrow therapeutic range for pancuronium and dictate stringent requirements for dosing accuracy and the necessity for meticulous monitoring of its effects. The relationships between the ranges of effective and lethal doses of pancuronium are presented graphically in Figure 2.

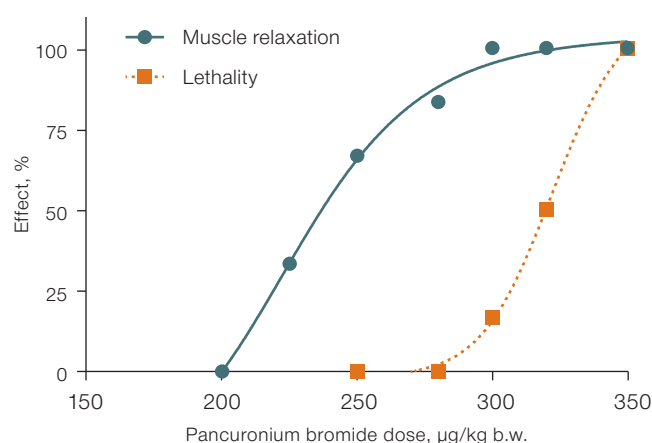


Figure prepared by the authors

Fig. 2. Dose-effect and dose-lethality relationships following single subcutaneous administration of pancuronium bromide to intact rats

Determination of effective and toxic doses of pancuronium bromide in a rat model of fenthion poisoning

According to preliminary toxicometric studies, the LD_{50} value of fenthion following a single subcutaneous administration in rats was 479.4 [465.3; 493.9] mg/kg. Animal deaths were recorded within the range of 6–24 h, with a median value of 11 h. The external signs of intoxication were represented by a set of cholinergic crisis symptoms (chromodacryorrhea, hypersalivation, incontinence, diarrhea, trismus, myofibrillations) and myasthenic syndrome, which appeared 15–30 min after administration and persisted for at least seven days. The residual activity of blood plasma acetylcholinesterase (AChE) in rats 12 h after pathology induction ranged 45.0–55.0% of the baseline level.

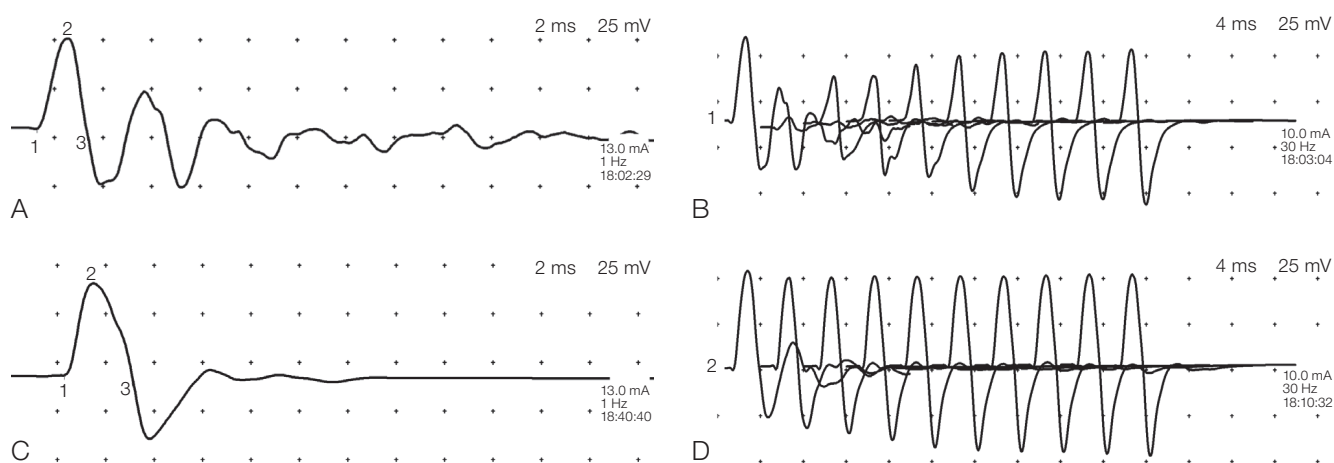


Figure prepared by the authors

Fig. 3. Changes in M-responses of the rat gastrocnemius muscle 12 h after fenthion administration: single stimulation (A) and rhythmic stimulation (B); reduction in the number and duration of repeated M-responses (C); restoration of the decrement-increment pattern to a normal M-response series (D): X-axis — M-response duration, ms; Y-axis — M-response amplitude, mV

Table 2. Results of assessing the effects of single subcutaneous administration of pancuronium bromide in rats during the acute phase of fenthion poisoning

Pancuronium dose, $\mu\text{g/kg bw}$	Reduction in repeated M-responses, %	Normalization of decrement-increment, %	Decrement detection frequency, %	Increase in muscle strength, %	Mortality rate, %
64.1	0.0	0.0	0.0	0.0	0.0
83.3	16.7	66.7	0.0	33.3	0.0
108.3	83.3	100.0	0.0	100.0	0.0
140.8	100.0	66.7	33.3	66.7	33.3
183.0	100.0	0.0	100.0	16.7	83.3
238.0	100.0	0.0	100.0	0.0	100.0

Table compiled by the authors based on original data

The neurophysiological profile of fenthion poisoning corresponded to the pattern previously described in a rat model of dimethoate poisoning [14]. In total, 12 h after fenthion administration, all animals exhibited numerous repeated M-responses to single stimulation, and in 80.0% of cases, a pronounced decrement-increment pattern was observed based on rhythmic stimulation data (Fig. 3A, B).

Administration of pancuronium bromide during the acute phase of poisoning at doses of 83.3 and 108.3 $\mu\text{g/kg bw}$ contributed to the improvement of NMT, manifesting as restored muscle strength according to CFTs, a reduced number of repeated M-responses, and normalization of the response to rhythmic stimulation. From a neurophysiological perspective, the recorded changes should be interpreted as a reduction in muscle hyperexcitability, restoration of nerve impulse discreteness, and, ultimately, an increase in the reliability of NMT. It was established that after administration

of pancuronium bromide at a dose of 108.3 $\mu\text{g/kg bw}$, ENMG data indicated a decrease in the number of repeated M-responses, as well as restoration of the decrement-increment pattern to a normal M-response series (Fig. 3C, D).

Based on CFT data and neurophysiological monitoring, the range of effective doses of pancuronium for correcting NMT impairments in the acute phase of fenthion poisoning in rats was determined (Table 2). The efficacy criteria were the electrophysiological signs of NMT normalization described above and the restoration of muscle strength within 1 h after pancuronium bromide administration. Administration of pancuronium at doses exceeding 140.8 $\mu\text{g/kg bw}$ resulted in an increased severity of NMB, manifesting as a decremental response instead of a decrement-increment pattern and progression of the myasthenic syndrome up to respiratory muscle paralysis.

The calculated ED_{50} value of pancuronium bromide, when administered during the acute phase of severe fenthion poisoning, was significantly lower than that in intact rats, amounting to 90.1 [77.3; 105.1] $\mu\text{g/kg}$ ($\text{ED}_{16} = 73.4 \mu\text{g/kg}$, $\text{ED}_{84} = 113.4 \mu\text{g/kg}$). A leftward shift of the lethality curve was also observed against the background of fenthion poisoning: $\text{LD}_{50} = 152.3$ [130.6; 177.6] $\mu\text{g/kg}$ ($\text{LD}_{16} = 124.0 \mu\text{g/kg}$, $\text{LD}_{84} = 191.6 \mu\text{g/kg}$). Graphs illustrating these relationships are presented in Figure 4. The therapeutic index of subcutaneously administered pancuronium bromide against the background of fenthion poisoning was 1.69, and the value of the certain safety factor was 0.59.

Thus, the necessity of reducing the effective dose by 2.64 times to correct the myasthenic syndrome, as well as maintaining a narrow therapeutic range of this medication, has been established. Moreover, the calculated ED_{50} value of pancuronium bromide for intact animals exceeds its LD_{99} value for rats under severe fenthion poisoning conditions.

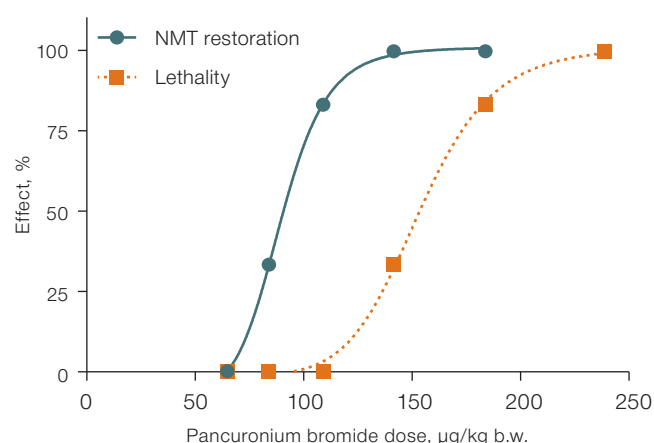


Figure prepared by the authors

Fig. 4. Dose-effect and dose-lethality curves obtained after subcutaneous administration of pancuronium bromide to rats 12 h after fenthion poisoning

² Kryukova EV, editor. Military Field Therapy: National Guide. 2nd ed., revised and expanded. Moscow: GEOTAR-Media; 2023.

DISCUSSION

Modern approaches to the treatment of anticholinesterase poisoning involve the use of atropine, oximes, and anticonvulsant drugs² [1, 3, 9]. Despite a long history of investigating the pharmacological potential of muscle relaxants in anticholinesterase poisoning, no validated approaches for their use as part of antidotal therapy have been established to date. This is due to a number of reasons, which, in our opinion, are related to specific features of the design of the conducted studies.

The scientific literature presents isolated clinical studies describing the experience of administering tubocurarine, pancuronium, and pipecuronium in organophosphorus (OP) poisoning [11, 12, 15, 17]. In particular, the successful use of pancuronium bromide administered intravenously at doses of 0.5 mg [16] and 1.0 mg [15] was reported, which is 5–10 times lower than the recommended doses for achieving muscle relaxation. However, the data provided in these studies are not analytical in nature, preventing the determination of fundamental principles for dosing and assessing drug effects, considering their low safety margin. Our study is among those few performed using an *in vivo* model, which enabled the evaluation of complex interactions between OPs and muscle relaxants at the organismal level through clinical and instrumental monitoring of NMT impairments and provided a detailed rationale for the effective doses of the drug.

According to Besser et al. [15], the phenomena of decrement-increment and repeated M-responses reflect different degrees of AChE inhibition severity, which was also confirmed by our studies [14]. These phenomena are caused by two main electrophysiological mechanisms mediated by nicotinic acetylcholine receptors (nAChRs). In contrast, complete suppression of NMT, manifested as a decremental response, is caused by desensitization of postsynaptic nAChRs due to a depolarizing block [18]. Stimulus-induced antidromic backfiring (SIAB) is attributed to excessive stimulation of axonal presynaptic nAChRs, which mediate autofacilitation of neurotransmitter release, under conditions of incomplete AChE inhibition and the absence of a desensitization block in postsynaptic nAChRs.

Studies suggest that precisely this mechanism, which underlies the decrement-increment response to rhythmic stimulation and spontaneous myofibrillations, can be blocked by low concentrations of D-tubocurarine or pancuronium, insufficient to block postsynaptic nAChRs [18, 19]. Repeated M-responses (repetitive compound muscle action potentials) following a single supramaximal stimulus are caused by an increased duration of interactions between acetylcholine and postsynaptic nAChRs, as well as lateral diffusion of an excess amount of the neurotransmitter, leading to the activation of several adjacent receptors. Collectively, this results in prolonged end-plate potentials and impaired discreteness of the nerve impulse [14, 15, 20]. The differing sensitivity

of pre- and postsynaptic nAChRs to the action of muscle relaxants, described in [15], was confirmed in our studies. For instance, restoration of the decrement-increment pattern to a normal M-response series was observed upon administration of lower pancuronium doses than those required to suppress repeated M-responses.

Pre- and postsynaptic nAChRs remain potential therapeutic targets at the neuromuscular junction (NMJ) for their competitive antagonists in OP intoxication. In this context, attempts to restore NMB through the application of muscle relaxants appear logical and justified. The concept behind their use in this situation is straightforward: it is necessary to administer a dose of a muscle relaxant that is sufficient to counteract the effects of excessive acetylcholine stimulation of nAChRs and leads to the normalization of NMJ function. Our studies have confirmed the hypothesis of Sheridan et al. that the dose of a muscle relaxant should not be sufficiently high to independently induce a non-depolarizing NMB in the “normalized” NMJ due to excessive antagonism [21].

According to Karalliedde, a marked increase in sensitivity to muscle relaxants should be anticipated in IS [22], analogous to cases of reduced vecuronium doses during the recovery phase from a depolarizing block induced by suxamethonium [23]. The results of our study support these hypotheses, demonstrating that the median effective dose of pancuronium in OP poisonings must be reduced by at least a factor of 2.6 compared to the median muscle-relaxing dose in intact animals. Furthermore, we observed a leftward shift in the dose-effect and dose-lethality curves while the narrow therapeutic range of the drug was maintained within the same limits.

Further research on extrapolating the obtained data on therapeutic doses of pancuronium bromide to humans in cases of OP poisoning is of significant interest. The recommended median effective doses for clinical use are 60.0–100.0 µg/kg upon intravenous administration [24], which is 2.4–4.0 times lower than the established analogous dose for rats (238.0 µg/kg). This fact indicates a comparable sensitivity to pancuronium between rats and humans, considering the traditionally accepted dose conversion based on body weight and surface area, thereby confirming the external validity of the selected experimental model. A more detailed analysis of preclinical data for their translation into clinical practice constitutes an independent task and represents a future direction of this scientific work.

It is widely acknowledged that administering muscle relaxants to patients who already require mechanical ventilation due to the acute respiratory failure typical of a cholinergic crisis (primarily of central origin) would help prevent subsequent excessive nAChR stimulation and NMJ dysfunction, without significantly altering the treatment strategy [11, 17, 21]. On the basis of this rationale, the authors conducted what they claimed to be the first pilot randomized clinical trial assessing the impact of muscle relaxants on the duration of the intubation period in

patients with OP poisonings [12]. During prolonged intravenous infusion of high-dose rocuronium (1.5 mg/kg/h), the researchers observed a predictable increase in the intubation period compared to the control group. The study design and its results are debatable due to the insufficiently justified selection of rocuronium doses, the lack of dynamic neurological examination, and the failure to assess expected endpoints such as the development of IS, critical illness polyneuropathy/myopathy, or delayed organophosphate-induced polyneuropathy, since the anticipated effects of muscle relaxants are precisely the prevention of these pathological conditions.

It should be mentioned that priority in this direction belongs to Russian researchers who published a similar study over 20 years ago [11]. That study demonstrated that the use of relaxant doses of pipecuronium (0.01–0.015 mg/kg intravenously, drip infusion) as part of a comprehensive therapy for karbofos (malathion) poisoning, which significantly reduced the severity of toxic neuromyopathy. This was evidenced by a faster recovery of muscle strength, a lower frequency and duration of peripheral respiratory paralysis, and a lower average number of hospital bed-days and mortality rates. The authors emphasized the importance of prolonged administration of muscle relaxants, selecting their optimal doses and administration regimens, considering the possibility of early administration at the prehospital stage in cases of OP poisoning.

The potential for prolonging the action of muscle relaxants was demonstrated by Iwasaki et al. in a small-size clinical study investigating the characteristics of the muscle relaxant effect of pancuronium following subcutaneous administration in patients [25]. It was established that subcutaneous administration in the upper limb region did not differ from intravenous administration in terms of the intensity and speed of the onset of the effect, as measured by acceleromyography in the “train-of-four” (TOF) mode. However, injection in the ankle region resulted in a significantly slower onset and prolonged duration of the muscle relaxant effect, the intensity of which was lower than with intravenous administration at an equal dose. On this basis, we specifically selected subcutaneous administration of pancuronium in rats to achieve an increased duration of effect without the need to induce deep NMB.

The obtained results underscore the necessity for precise calculation of muscle relaxant doses in cases of OP poisoning and the simultaneous monitoring of NMT status. The use of ENMG for this purpose is recognized as the most accurate and should be considered the “gold standard” [26, 27]. However, several publications have reported data indicating a good correlation between “train-of-four” (TOF) stimulation values determined by acceleromyography (AMG), mechanomyography (MMG), and ENMG. Although AMG is less accurate in assessing the depth of NMB compared to ENMG and MMG, it is the most widely used

method in routine clinical practice due to its ease of use and applicability across various muscles [28]. The authors of these studies are unanimous in concluding that the choice of a specific monitoring method is less critical than the very fact of its presence and proper execution, since any of these methods allows for an objective assessment of NMT status. Furthermore, NMB monitoring is essential in cases of OP poisoning for the timely diagnosis of peripheral nervous system impairments.

CONCLUSION

In this study, we have determined experimentally electrophysiological criteria for correcting NMT impairments — underlying IS in AChE poisonings — using pancuronium bromide. These criteria specifically include a reduction in the number of repeated M-responses and a change in the pattern of the M-response series during high-frequency rhythmic stimulation from a decremental to a decrement-increment pattern. The regression of the NMB, as evidenced by this dynamic change in the ENMG profile, was further supported by an increase in muscle strength according to two CFTs and a reduction in the mortality rate among the experimental groups.

An experimental rationale for the range of effective (therapeutic) doses of pancuronium bromide in cases of OP poisoning has been provided, and the persistence of its narrow therapeutic range under conditions of pronounced AChE inhibition has been determined. Subcutaneous administration of pancuronium bromide at low doses (2.6 times lower than the median effective dose for intact animals) was found to alleviate severe NMT impairments in cases of severe OP poisoning through partial blockade of both pre- and postsynaptic nAChRs.

The presented data support the hypothesis that the use of muscle relaxants effectively mitigates the manifestations of OP-induced myasthenic syndrome. Given that virtually all patients with severe OP poisoning require mechanical ventilation as part of the standard treatment protocol, the early and prolonged administration of pancuronium bromide, a long-acting muscle relaxant, at low doses appears justified and promising for the prevention of intermediate syndrome. It is important to note that the dosing of muscle relaxants can be controlled in real-time using MMG or AMG modules to assess neuromuscular transmission and perform tests in the following sequence: single stimulation — train-of-four (TOF) — post-tetanic count (PTC). However, in order to develop robust clinical recommendations, further studies are needed to evaluate the efficacy of different muscle relaxants within antidotal therapy regimens, especially in combination with cholinesterase reactivators and atropine, under the condition of administering these drugs during different periods of intoxication.

References

- Yudin MA, Chepur SV, Fedonyuk VP, Subbotina SN, Kolesnikov AM, Sazonova AV, et al. Comparative analysis of the creation of antidotes of organophosphorus toxic substances in Russia and abroad. *Military Medical Journal*. 2019;340(2):18–26 (In Russ.). EDN: [YWQQLB](#)
- Ivanov IM, Subbotina SN, Alkhutova IM, Yudin MA, Chepur SV. Mechanisms of respiratory disorders in case of poisoning with organophosphate compounds and directions of correction using bronchodilators. *Medline.ru*. 2022;23:185–200 (In Russ.). EDN: [LBYUOZ](#)
- Amend N, Niessen KV, Seeger T, Wille T, Worek F, Thiermann H. Diagnostics and treatment of nerve agent poisoning—current status and future developments. *Annals of the New York Academy of Sciences*. 2020;1479(1):13–28. <https://doi.org/10.1111/nyas.14336>
- Bird SB, Krajacic P, Sawamoto K, Bunya N, Loro E, Khurana TS. Pharmacotherapy to protect the neuromuscular junction after acute organophosphorus pesticide poisoning. *Annals of the New York Academy of Sciences*. 2016;1374(1):86–93. <https://doi.org/10.1111/nyas.13111>
- Tyunin MA, Chepur SV, Gogolevsky AS, Selivanov DV, Burtseva NI, Ilyinskiy NS. The problem of the intermediate syndrome following poisoning with anti-cholinesterase agents. *Toxicological Review*. 2017;4:40–9 (In Russ.). <https://doi.org/10.36946/0869-7922-2017-4-40-49>
- Leon-S FE, Pradilla-A G, Amboa N, Vesga E. Multiple system organ failure, intermediate syndrome, congenital myasthenic syndrome, and anticholinesterase treatment: the linkage is puzzling. *Journal of Toxicology: Clinical Toxicology*. 1996;34(2):245–7. <https://doi.org/10.3109/15563659609013779>
- Çolak Ş, Erdoğan MÖ, Baydin A, Afacan MA, Kati C, Duran L. Epidemiology of organophosphate intoxication and predictors of intermediate syndrome. *Turkish Journal of Medical Sciences*. 2014;44(2):279–82. <https://doi.org/10.3906/sag-1211-31>
- Zakurdaev VV. Toxic myopathy in acute FOS intoxication. *Military Medical Journal*. 1986;10:49–51 (In Russ.).
- De Bleecker J, Van Den Neuoker K, Willens J. The intermediate syndrome in organophosphate poisoning: presentation of a case and review of the literature. *Journal of Toxicology: Clinical Toxicology*. 1992;30(3):321–9. <https://doi.org/10.3109/15563659209021546>
- Sedgwick EM, Senanayake N. Pathophysiology of the intermediate syndrome of organophosphorus poisoning. *Journal of Neurology, Neurosurgery and Psychiatry*. 1997;62(2):201–2. <https://doi.org/10.1136/jnnp.62.2.201>
- Bonitenko UU, Bonitenko EU, Pershin VN. Muscle relaxants and organophosphorus insecticide poisoning. *Medline.ru*. 2004;5(9):40–4 (In Russ.).
- Dhanarisi J, Shihana F, Harju K, Mohamed F, Verma V, Shahmy S, et al. A pilot clinical study of the neuromuscular blocker rocuronium to reduce the duration of ventilation after organophosphorus insecticide poisoning. *Clinical Toxicology*. 2020;58(4):254–61. <https://doi.org/10.1080/15563650.2019.1643467>
- Ilyinskiy NS, Tyunin MA, Matrosova MO. Methodological approaches to the assessment of paralytic syndrome of toxic genesis in experiments on rodents. *Laboratory Animals for Science*. 2021;3:71–5 (In Russ.). <https://doi.org/10.29296/2618723X-2021-03-09>
- Tyunin MA, Ilyinskiy NS, Gogolevskii AS, Kruchinin EG, Gladkikh VD, Matseichik VA, et al. Electrophysiological methods for the diagnosis of disorders of neuromuscular transmission in acute poisoning with organophosphorus compounds. *Military Medical Journal*. 2020;341(10):11–9 (In Russ.). EDN: [NSYNCD](#)
- Besser R, Gutmann L. Quantitative study of the pancuronium antagonism at the motor endplate in human organophosphorus intoxication. *Muscle & Nerve*. 1995;18(9):956–60. <https://doi.org/10.1002/mus.880180906>
- Singh G, Avasthi G, Khurana D, Whig J, Mahajan R. Neurophysiological monitoring of pharmacological manipulation in acute organophosphate (OP) poisoning. The effects of pralidoxime, magnesium sulphate and pancuronium. *Electroencephalography and Clinical Neurophysiology*. 1998;107(2):140–8. [https://doi.org/10.1016/s0013-4694\(98\)00053-4](https://doi.org/10.1016/s0013-4694(98)00053-4)
- Eddleston M, Chowdhury FR. Pharmacological treatment of organophosphorus insecticide poisoning: the old and the (possible) new. *British Journal of Clinical Pharmacology*. 2016;81(3):462–70. <https://doi.org/10.1111/bcp.12784>
- Besser R, Vogt T, Gutmann L, Wessler I. High pancuronium sensitivity of axonal nicotinic-acetylcholine receptors in humans during organophosphate intoxication. *Muscle & Nerve*. 1991;14(12):1197–201. <https://doi.org/10.1002/mus.880141210>
- Blaber L, Bowman WC. Studies on the repetitive discharges evoked in motor nerve and skeletal muscle after injection of anticholinesterase drugs. *British Journal of Pharmacology and Chemotherapy*. 1963;20:326–44. <https://doi.org/10.1111/j.1476-5381.1963.tb01472.x>
- Kumar RS, Kuruvilla A. Repetitive compound muscle action potentials in electrophysiological diagnosis of congenital myasthenic syndromes: a case report and review of literature. *Annals of Indian Academy of Neurology*. 2010;13(2):139–41. <https://doi.org/10.4103/0972-2327.64645>
- Sheridan RD, Smith AP, Turner SR, Tattersall JEH. Nicotinic antagonists in the treatment of nerve agent intoxication. *Journal of the Royal Society of Medicine*. 2005;98(3):114–5. <https://doi.org/10.1177/014107680509800307>
- Karalliedde L. Organophosphorus poisoning and anaesthesia. *Anaesthesia*. 1999;54(11):1073–88. <https://doi.org/10.1046/j.1365-2044.1999.01061.x>
- Feldman S, Fauvel N. Potentiation and antagonism of vecuronium by decamethonium. *Anesthesia and Analgesia*. 1993;76:631–4. <https://doi.org/10.1213/00000539-199303000-00033>
- Murray MJ, DeBlock H, Erstad B, Gray A, Jacobi J, Jordan C, et al. Clinical Practice Guidelines for Sustained Neuromuscular Blockade in the Adult Critically Ill Patient. *Critical Care Medicine*. 2016;44(11):2079–103. <https://doi.org/10.1097/CCM.0000000000002027>
- Iwasaki H, Namiki A, Omote T, Omote K. Neuromuscular effects of subcutaneous administration of pancuronium.

- The Journal of the American Society of Anesthesiologists*. 1992;76(6):1049–51.
<https://doi.org/10.1097/00000542-199206000-00026>
26. Nemes R, Lengyel S, Nagy G, Hampton DR, Gray M, Renew JR, et al. Ipsilateral and simultaneous comparison of responses from acceleromyography and electromyography-based neuromuscular monitors. *Anesthesiology*. 2021;135(4):597–611.
<https://doi.org/10.1097/ALN.0000000000003896>
27. Lee W. The latest trend in neuromuscular monitoring: return of the electromyography. *Anesthesia and Pain Medicine*. 2021;16(2):133–7.
<https://doi.org/10.17085/apm.21014>
28. Chubchenko NV, Lebedinskiy KM. Monitoring of various types of neuromuscular blockade. *Messenger of Anesthesiology and Resuscitation*. 2016;13(6):64–71 (In Russ.).
EDN: [XIBQXN](#)

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MECHANISMS OF HEAT STRESS POTENTIATION OF FENTANYL LETHALITY IN RATS

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Introduction. As a rule, preclinical safety assessment of narcotic analgesic agents is conducted at ambient temperatures of 20–24 °C. However, their clinical use may occur under different thermal conditions. Previous studies have demonstrated the ability of heat stress to potentiate the lethal and narcotic effects of fentanyl in rats, yet the mechanisms underlying this phenomenon remain unknown.

Objective. Testing hypotheses on the mechanisms of fentanyl toxicity potentiation in rats under heat stress conditions.

Materials and methods. The study was conducted on outbred male albino rats weighing 191–210 g. We investigated the effects of intravenous fentanyl administration at a dose of 200 µg/kg and/or a 40-min exposure to an ambient temperature of 40 °C on body temperature and mass, brain moisture content and mass, glutamine concentration in brain tissue, biochemical parameters of blood collected from the *a. carotis communis* and *v. jugularis interna*, and oxygen consumption by brain homogenates. Statistical analysis was performed using the OriginPro software.

Results. Fentanyl administration induced opisthotonus, coma, bradypnea, and fundal cyanosis. The 40-min lethality following fentanyl injection was 0–9% at an ambient temperature of 22 °C and 68–71% at an ambient temperature of 40 °C. In surviving rats removed from the thermal chamber, rectal temperature was elevated to 42.9 °C. The relative mass of the freshly isolated brain and the brain dried to a constant weight increased by 7.4% and 7.2%, respectively. Glutamine content in brain tissue increased by 46%. Plasma concentrations of ammonia, creatinine, and lactate were elevated by 2.0–2.2, 2.1–2.3, and 1.5–1.6 times, respectively. In the absence of fentanyl administration, no lethality was observed in rats placed in the thermal chamber. Rectal temperature increased to 42.7 °C. The relative mass of the freshly isolated brain and the dried brain increased by 6.1% and 8.9%, respectively. Brain glutamine content increased by 43%. Plasma creatinine levels rose by 2.2–2.4 times, and lactate levels increased by 25–45%. In the absence of heat stress, fentanyl increased plasma creatinine concentration only by 1.6–1.8 times. The arteriovenous gradient of plasma ammonia concentration was positive in all animals. Oxygen consumption by brain homogenates decreased by 10% under isolated heat stress and increased by 7% under a combined action of heat stress and fentanyl administration.

Conclusions. Hypoxemia, lactic acidemia, and hyperammonemia were necessary conditions for the aggravating effect of heat stress on acute fentanyl intoxication in rats. Conversely, irreversible thermal damage to biological tissues, organism dehydration, cerebral edema, swelling, hyperemia, and glutamine accumulation in the brain were not identified as such necessary conditions.

Keywords: hyperammonemia; brain tissue glutamine; lactic acidemia; lethality; acute intoxication; body temperature; heat stress; fentanyl

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МЕХАНИЗМЫ ПОТЕНЦИРОВАНИЯ ТЕПЛОВЫМ СТРЕССОМ ЛЕТАЛЬНОГО ДЕЙСТВИЯ ФЕНТАНИЛА НА КРЫС

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Введение. Доклиническое изучение безопасности наркотических анальгетиков проводят при температуре воздуха 20–24 °C, но их применение возможно и при иной температуре. Ранее показано потенцирование тепловым стрессом летального и наркотического действия фентанила на крыс, однако механизмы этого феномена неизвестны.

Цель. Проверка гипотез о механизмах повышения токсичности фентанила для крыс в условиях теплового стресса.

Материалы и методы. Исследование проведено на беспородных самцах крыс-альбиносов массой 191–210 г. Изучали влияние внутривенного введения фентанила в дозе 200 мкг/кг и (или) сорокаминутного пребывания при температуре воздуха 40 °C на температуру и массу тела, влагосодержание и массу головного мозга, содержание глутамина в ткани мозга, биохимические показатели крови из *a. carotis communis* и *v. jugularis interna* крыс, потребление кислорода гомогенатами их головного мозга. Статистический анализ проводили с использованием программного обеспечения OriginPro.

Результаты. Введение фентанила вызывало опистотонус, кому, брадипноэ и цианоз глазного дна. Летальность за 40 мин после введения фентанила составляла 0–9% при температуре воздуха 22 °C и 68–71% при температуре воздуха 40 °C. Ректальная температура у выживших крыс по извлечении из термокамеры была повышена до 42,9 °C, относительная масса свежееизвлеченного

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и высушенного до постоянной массы головного мозга — на 7,4 и 7,2% соответственно, содержание глутамина в его ткани — на 46%; в плазме крови повышалась концентрация аммиака в 2,0–2,2 раза, креатинина — в 2,1–2,3 раза и лактата — в 1,5–1,6 раза. Без введения фентанила летальность крыс в термокамере отсутствовала, ректальная температура повышалась до 42,7 °C, относительная масса свежеевлеченного головного мозга — на 6,1% и сухого — на 8,9%, содержание в нем глутамина — на 43%, уровень в плазме крови креатинина — в 2,2–2,4 раза и лактата — на 25–45%. Без теплового стресса фентанил лишь повышал в 1,6–1,8 раза концентрацию креатинина в плазме крови. Артериовенозный градиент концентрации аммиака в плазме крови у всех животных был положительным. Потребление кислорода гомогенатами головного мозга снижалось на 10% при изолированном тепловом стрессе и повышалось на 7% при тепловом стрессе на фоне введения фентанила.

Выводы. Гипоксемия, лактацидемия и гипераммониемия были необходимыми условиями отягчающего влияния теплового стресса на острую интоксикацию фентанилом у крыс. Необратимое термическое повреждение биотканей, обезвоживание организма, отек, набухание, гиперемия головного мозга или накопление в нем глутамина не являлись такими условиями.

Ключевые слова: гипераммониемия; глутамин ткани мозга; лактацидемия; летальность; острая интоксикация; температура тела; тепловой стресс; 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

As a rule, preclinical drug studies are conducted at an ambient temperature of 20–24 °C, as stipulated by the Guidelines for the Housing and Care of Laboratory Animals.¹ Consequently, the influence of heat stress on the biological activity of a number of pharmaceuticals is poorly represented in the scientific literature, despite its potential role as a background condition in real-world use, particularly in prehospital settings. This underscores the relevance of investigating the biological activity of drugs under climatic conditions that promote body hyperthermia.

We have previously demonstrated [1] that intravenous administration of fentanyl to rats at doses not exceeding the LD₅ (at an ambient temperature of 20–24 °C) becomes uniformly lethal upon subsequent 40-min exposure to an ambient temperature of 40 °C, a condition that is well-tolerated in the absence of fentanyl. In order to explain this previously unknown phenomenon, hypotheses suggesting hyperammonemia and the resulting brain swelling as mechanisms for the aggravating effect of heat stress on acute fentanyl intoxication were advanced.

The aim of this study was to test the above hypotheses regarding the mechanisms of fentanyl toxicity enhancement in rats under heat stress conditions.

MATERIALS AND METHODS

The study was conducted from January to May 2025 on outbred male albino rats (191–210 g) obtained from

the Rappolovo Laboratory Animal Nursery (Kurchatov Institute). Two experimental series were performed.

In the first series of experiments ($n = 77$), we investigated the effects of isolated or combined exposure to fentanyl and heat stress on rectal temperature, body and brain mass, brain glutamine content, and biochemical parameters of blood collected from the *a. carotis communis* and *v. jugularis interna*. The study design and animal distribution into experimental groups are presented in Table 1.

In the second series of experiments, a separate cohort of rats ($n = 37$), comparable to those used in the first series, was employed to investigate the effects of isolated or combined exposure to fentanyl and heat stress on oxygen consumption by brain homogenates. The study design is presented in Table 2.

A fentanyl solution of 50 µg/mL (Moscow Endocrine Plant, batch 30212) was administered to the animals at a volume of 4 mL/kg via the lateral tail vein. The fentanyl dose was 200 µg/kg, which, according to previous data [1], corresponded to LD_{5/24 h} at 22 °C and LD_{70/24 h} at 40 °C over 40 min, followed by maintenance at 22 °C.

Thermal stress was simulated in a BMT Stericell SC 111 ECO thermal chamber (Czech Republic) with a volume of 111 L. The following environmental parameters were maintained: air temperature at 40 ± 1 °C, relative humidity at 48%, and an air exchange rate of 45 chamber volumes per hour. During their stay in the thermal chamber, all animals were placed in restrainers made of perforated aluminum. Rats that did not receive fentanyl and were kept in restrainers at room temperature were considered intact (control) animals.

¹ GOST 33216-2014 "Guidelines for the Housing and Care of Laboratory Animals. Requirements for the Maintenance and Care of Laboratory Rodents and Rabbits".

Table 1. Protocol for the assessment of fentanyl and/or heat stress effects on body temperature, body and brain mass, glutamine content in dry brain tissue, and biochemical parameters of blood from the common carotid artery and internal jugular vein in rats

Number of rats surviving until examination	Exposure of rats		Parameters
	Pharmacological	Microclimatic	
11 out of 11	absent	22 °C for 40 min post-injection	body mass and temperature, brain mass, glutamine content in dry brain tissue, and levels of ammonia, creatinine, and lactic acid in blood from the <i>a. carotis communis</i> and <i>v. jugularis interna</i> at 40 min post-injection
10 out of 11	fentanyl		
10 out of 10	absent	40 °C for 40 min post-injection	
13 out of 45	fentanyl		

Table compiled by the authors

Table 2. Protocol for the assessment of fentanyl and/or heat stress effects on oxygen consumption by rat brain homogenates

Number of rats surviving until examination	Exposure of rats		Parameters
	Pharmacological	Microclimatic	
6 out of 6	absent	22 °C for 40 min post-injection	oxygen consumption by brain homogenates extracted 40 min after injection
6 out of 6	fentanyl		
6 out of 6	absent	40 °C for 40 min post-injection	
6 out of 19	fentanyl		

Table compiled by the authors

Body temperature was measured with a precision of 0.1 °C using an electric thermometer with a rat-specific probe (RET-2, WPI, China). The probe tip was inserted 3 cm into the rectum. Body weight was measured with a precision of 1g both before and 40 min after administration of the solutions to the animals. The color of the ocular fundus was assessed indirectly by observing the eye color in albino rats, which lack pigments other than hemoglobin.

In the first series of experiments, rats were immobilized in the dorsal position for blood collection; those not receiving fentanyl were subjected to light halothane anesthesia. Blood samples of 0.5–1 mL were collected from the *a. carotis communis* and *v. jugularis interna*. Plasma was separated by centrifugation at 4 °C for 10 min at 3000 rpm (5400 g). Using an A-25 biochemical analyzer (BioSystems, Spain), the following parameters were measured:

- Ammonia — using an Ammonia Ultra reagent kit (Sentinel Diagnostics, Italy);
- Creatinine — via the reaction with picric acid, using a Creatinine-Novo-A reagent kit (Vector-Best, Russia);
- Lactic acid — using a Lactate Vital reagent kit (Vital Development Corporation, Russia).

Following the completion of blood collection, the animals were decapitated; the brain was removed and weighed to the nearest 0.01 g both before and after

drying to constant weight at 105 °C. The dried brain was ground to a powder, suspended in nine parts of 0.01 M phosphate buffered saline (pH = 7.0), mixed, and centrifuged at 20,000 rpm (36,000 g) for 1 h at 4 °C. The supernatant was incubated for 10 min at 100 °C in the presence of sulfuric acid at a final concentration of 0.17 M. Ammonia was measured in the supernatant both before and after incubation using the method described above. Glutamine concentration in the tissue extract was determined as the difference between ammonia concentrations in the supernatant before and after acid hydrolysis [2]. An L-glutamine standard (Merck, Germany) was used to construct the calibration curve. Glutamine content in dry tissue was calculated in $\mu\text{mol/g}$.

In the second series of experiments, the brain was removed, weighed, and homogenized in cold Hank's solution (free of phenol red) to produce a uniform suspension, with a ratio of one mass unit of wet tissue to four mass units of Hank's solution. One milliliter of the resulting 20% homogenate was added to manometric vessels containing 2 mL of Hank's solution (without phenol red) in the main chambers and 0.2 mL of 10% potassium hydroxide solution in the central compartments.

Oxygen consumption was measured using the manometric method [3] with a Warburg apparatus (model Wa 0110, Glaswerke Ilmenau, GDR) at 38 °C and a shaker speed of 120 min^{-1} ; air served as the gas phase.

Manometer readings were recorded after a 10-min temperature equilibration period, then at 10-min intervals over a 70-min span. Oxygen consumption was expressed in microliters per gram of wet tissue, cumulatively.

Statistical analysis was performed using the OriginPro software. Results were presented as the mean value and its standard error ($M \pm m$). To test the normality of the distribution, the Shapiro–Wilk test was used. The effect of the applied interventions on parametric parameters was assessed using analysis of variance (ANOVA). When the obtained models were statistically significant, post-hoc comparisons of mean values between groups were conducted using Tukey's honest significant difference (HSD) test. The critical significance level α was set at 0.05.

RESULTS

Within several seconds after fentanyl administration, rats developed opisthotonus, tail extension, brief apnea followed by shallow, infrequent breathing, and pronounced cyanosis of the ocular fundus. The absence of auditory-motor and pain reflexes allowed the animals' neurological status to be classified as coma.

In the first series of experiments, the mortality rate among rats that received fentanyl over a 40-min period at 22 °C was 9%, while at 40 °C it reached 71%. For rats that did not receive fentanyl, a 40-min exposure to 40 °C was non-lethal. Thus, upon removal from the thermal chamber, their behavior appeared normal, and the ocular fundus exhibited a bright crimson color.

In surviving rats, the baseline rectal temperature was 38.0 ± 0.1 °C ($n = 44$). In total, 40 min after fentanyl administration, the rectal temperature of animals maintained at 22 °C decreased to 33.9 ± 0.2 °C ($n = 10$), while that of animals placed in the thermal chamber increased to 42.9 ± 0.3 °C ($n = 12$). Exposure to the thermal chamber

without fentanyl administration increased rectal temperature to 42.7 ± 0.3 °C ($n = 10$). These changes are presented in Fig. 1A.

Within 40 min after fentanyl injection, the body mass of rats kept at 22 °C decreased from 203 ± 5 g to 198 ± 5 g, with a similar trend observed in intact animals. Exposure to the thermal chamber without fentanyl administration reduced body mass from 200 ± 4 g to 190 ± 4 g, while in survivors receiving fentanyl, it decreased from 201 ± 5 g to 192 ± 5 g. Changes in body mass are shown in Fig. 1B.

The absolute mass of the isolated brain, as well as its ratio to the initial body mass of rats that received fentanyl and/or underwent heat stress, did not differ significantly from those in intact animals. The brain-to-body mass ratio 40 min after fentanyl administration and/or placement in the thermal chamber showed a tendency to increase in all groups compared to the intact group. For animals placed in the thermal chamber after fentanyl administration, this tendency was significant and manifested as a 7.4% increase in their relative brain mass compared to that in intact rats (Fig. 2A).

In intact rats, brain moisture content averaged 79.4%. Under isolated fentanyl exposure, it was 79.2%; under isolated hyperthermia, 78.9%; and under combined hyperthermia and fentanyl exposure, 79.5%. The intergroup differences were not statistically significant. The relative mass of the dry brain following exposure to fentanyl and heat stress exceeded that in intact rats by 7.2%, and after isolated heat exposure, by 8.9% (Fig. 2B).

In the extract of dry brain tissue, the concentration of free ammonia was below the detection limit of the method ($15 \mu\text{mol/L}$). However, following acid hydrolysis, it was detected at a level equivalent to the glutamine content. In intact rats, the glutamine concentration in dry brain tissue was $21.8 \pm 1.5 \mu\text{mol/g}$, which corresponded to $4.5 \mu\text{mol/g}$ in wet tissue and was consistent with the data published in [4]. In dry brain tissue subjected to isolated

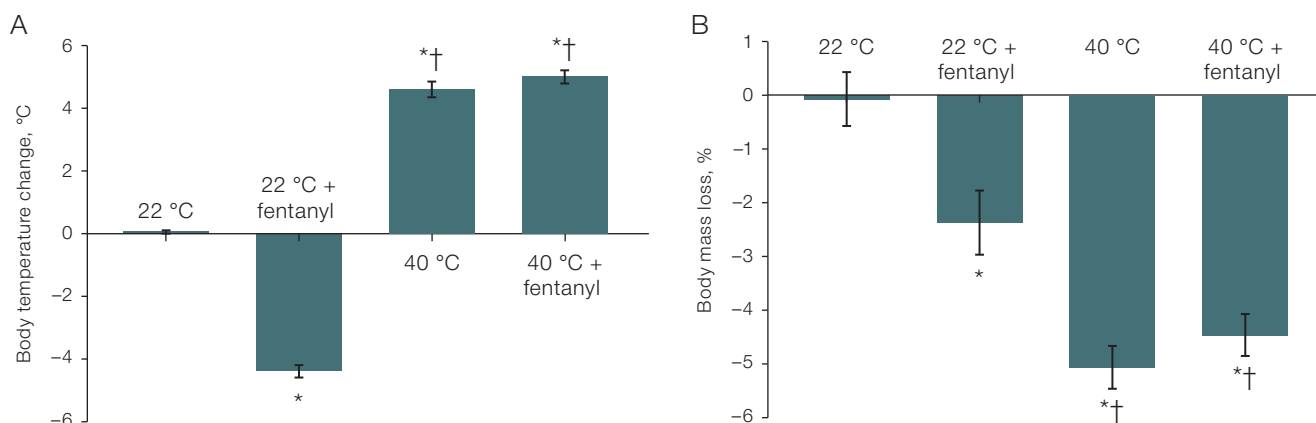
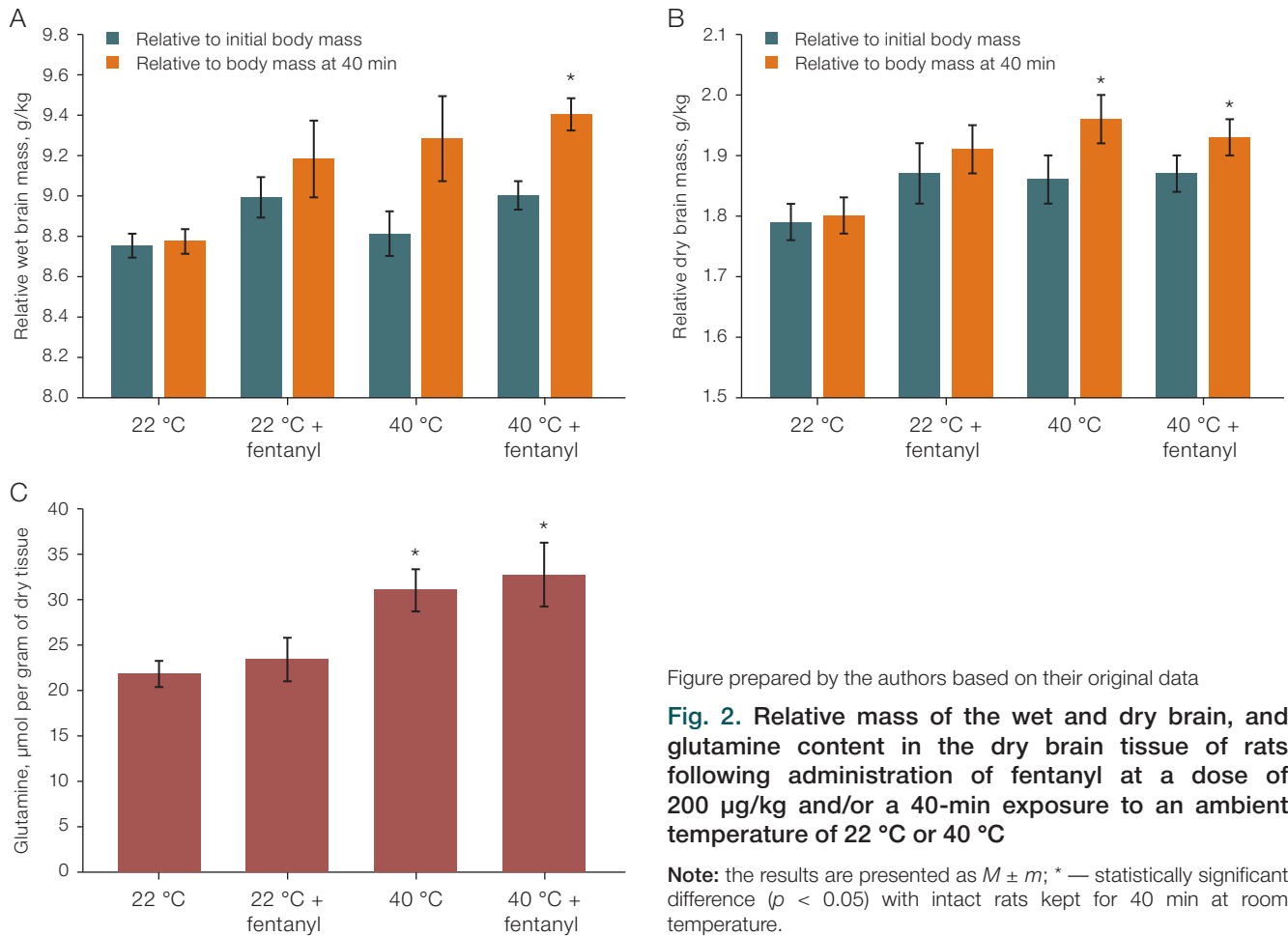


Figure prepared by the authors based on their original data

Fig. 1. Changes in rectal temperature and body mass of rats following administration of fentanyl at a dose of 200 $\mu\text{g/kg}$ and/or a 40-min exposure to an ambient temperature of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; statistically significant difference $p < 0.05$: * — with intact rats kept for 40 min at room temperature; † — with rats that received fentanyl and were kept for 40 min at room temperature.



heat stress or its combination with fentanyl exposure, the glutamine content was increased by 43% and 46%, respectively, compared to intact animals (Fig. 2C).

In the setting of combined fentanyl and heat stress exposure, the blood ammonia level was elevated by 2–2.2 times compared to intact animals. The value of the arteriovenous gradient of plasma ammonia concentration was positive in all animals. Under isolated heat stress, its value of 47 ± 13 µM was equal to that in intact rats, while under isolated fentanyl exposure or in combination with hyperthermia, it showed a tendency to increase: 87 ± 29 and 61 ± 18 µM, respectively (Fig. 3A).

The concentration of creatinine in the plasma of arterial and venous blood exceeded that in intact rats by 1.6–2.4 times in all experimental groups. This increase was the greatest (2.2–2.4-fold) under conditions of heat stress (Fig. 3B).

In the setting of heat stress, the level of lactic acid in the plasma of arterial and venous blood was 25–45% higher than in intact rats, and under hyperthermia combined with fentanyl administration, it was 50–60% higher. The lactate content in arterial blood exceeded its concentration in venous blood in all animals except those subjected to isolated heat stress (Fig. 3C).

In the second series of experiments, isolated exposure to fentanyl or heat stress was non-lethal, while their

combination resulted in the death of 13 (68%) out of 19 rats before removal from the thermal chamber.

The respiratory rate of brain homogenates remained stable across all experimental groups, ensuring a linear dynamic of oxygen consumption volume. For intact animals, it was described by the linear regression equation $y = 3.83 x$; for isolated fentanyl exposure — $y = 3.71 x$; for isolated heat stress — $y = 3.41 x$; and for the combined action of these agents — $y = 4.09 x$, where x is the incubation duration (min) and y is the specific volume of oxygen consumed (µL per 1 g of tissue). The mean oxygen consumption over 70 min was 3.8 µL/(g × min) in the intact group. Under isolated heat stress, it was 10% lower, and under heat stress combined with fentanyl action, it was 7% higher than in intact rats (Fig. 4).

DISCUSSION

In the thermal chamber, the rectal temperature of the rats increased, indicating the development of heat stress. Contrary to the hypothermic effect of fentanyl observed in the present study and described in the literature [5], after 40 min in the thermal chamber, the body temperature of rats that received fentanyl, as well as those under isolated heat exposure, approached 43 °C. This is known to be a threshold for irreversible tissue damage

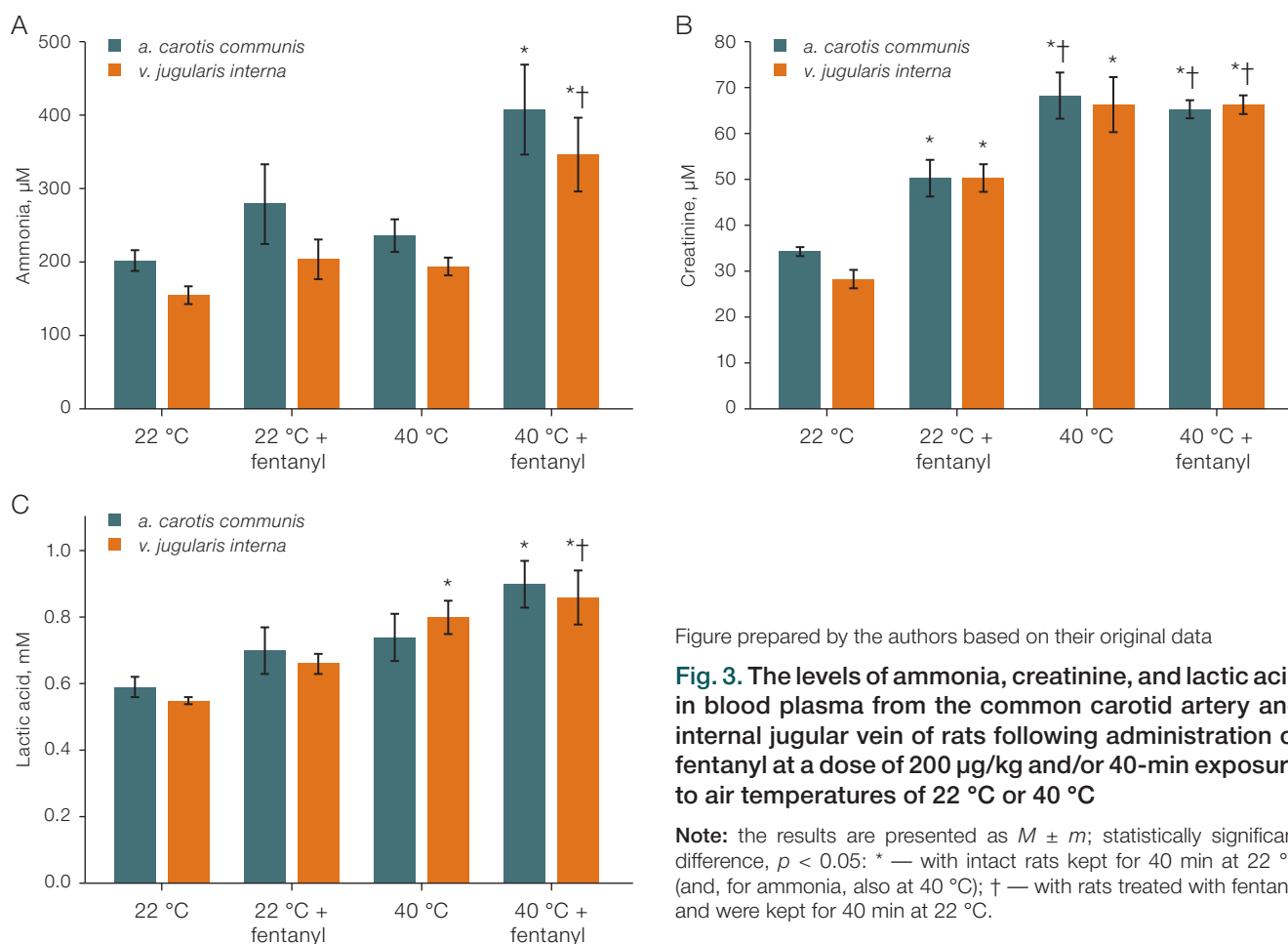


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Fig. 3. The levels of ammonia, creatinine, and lactic acid in blood plasma from the common carotid artery and internal jugular vein of rats following administration of fentanyl at a dose of 200 $\mu\text{g/kg}$ and/or 40-min exposure to air temperatures of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; statistically significant difference, $p < 0.05$: * — with intact rats kept for 40 min at 22 °C (and, for ammonia, also at 40 °C); † — with rats treated with fentanyl and were kept for 40 min at 22 °C.

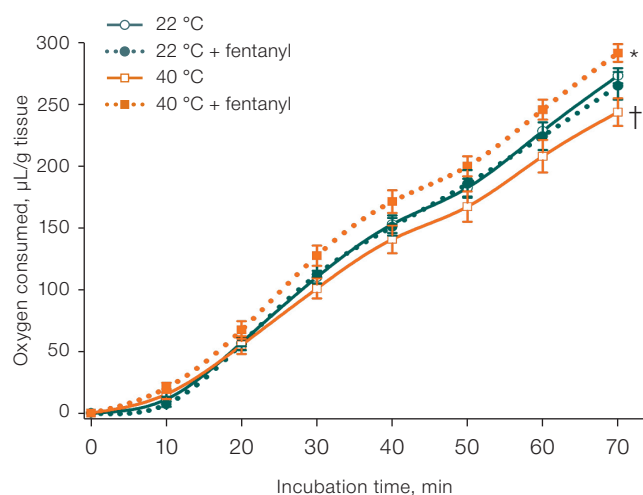


Figure prepared by the authors based on their original data

Fig. 4. Oxygen consumption by brain homogenates following administration of fentanyl at a dose of 200 $\mu\text{g/kg}$ to rats and/or their 40-min exposure to an ambient temperature of 22 °C or 40 °C

Note: the results are presented as $M \pm m$; statistically significant difference, $p < 0.05$: * — with intact rats left at room temperature for 40 min; † — rats treated with fentanyl and left for 40 min at an air temperature of 40 °C.

[6]. However, lethality was observed only in the setting of fentanyl administration, and, therefore, it was not caused by thermal damage to tissues or by a decrease in hemoglobin affinity for oxygen with increasing body temperature. For the same reason, lethality was not determined by organism dehydration: both water loss, leading to a decrease in body mass, and hemoconcentration, manifested by the accumulation of creatinine in the blood, were comparable in animals that received and did not receive fentanyl. The increase in blood viscosity due to dehydration could impede blood supply to the brain; however, in the absence of fentanyl, it was not lethal.

The hypothesis suggesting the contribution of brain swelling to the aggravating effect of heat stress on acute fentanyl intoxication was not experimentally confirmed. In rats that survived 40 min after the onset of the combined exposure to these factors, the increase in the relative mass of the wet and dry brain was comparable (by 7.4% and 7.2%, respectively), and the moisture content did not change significantly. This indicates the absence of pronounced cerebral edema, swelling, or hyperemia in these animals.

Under heat stress, brain dehydration was less pronounced than systemic dehydration, as evidenced by an increase in its relative mass while the absolute mass remained stable. Given that the mass of biological tissue

is positively correlated with its volume, the ratio of brain volume to cranial cavity volume during heat exposure could have increased, potentially elevating intracranial pressure. In accordance with Pascal's law, this excess hydraulic pressure, if transmitted to blood vessels, would impede cerebral blood flow. However, the comparable extent of increased relative brain mass in rats that did and did not receive fentanyl prior to placement in the thermal chamber — coupled with survival only in the latter group — does not support elevated intracranial pressure as a key mechanism in thanatogenesis.

Under the influence of fentanyl and/or heat stress, glutamine accumulated in the brain. Calculated per 1 g of wet tissue, its mean group content was higher than in intact rats by 0.2, 1.0, and 1.0 μmol at 40 min after fentanyl administration, placement in the thermal chamber, or a combination of these factors, respectively. Since postmortem synthesis of glutamine in brain tissue is impossible due to the cessation of oxidative phosphorylation within 10.5 s after decapitation [7], this increase reflected the direction of in-vivo changes in the primary reaction of ammonia detoxification by the brain: the synthesis of glutamine by astrocytic glutamine synthetase [8]. The comparable extent of glutamine accumulation in the brains of rats that did and did not receive fentanyl prior to placement in the thermal chamber allows this disturbance to be excluded from being a lethal factor.

A positive arteriovenous transcerebral gradient of plasma ammonia concentration was observed in all rats, which is consistent with the data reported in [8] and indicates that the primary source of glutamine in brain tissue was ammonia extracted from the blood. Each milliliter of blood from intact animals or those subjected to isolated heat stress lost 47 nmol of ammonia during its passage from the common carotid artery to the internal jugular vein. Under isolated fentanyl exposure, this loss amounted to 87 nmol, and under combined fentanyl and heat stress, it was 61 nmol. The volumetric rate of cerebral blood flow in rats is estimated at 1.3 mL/(g $\times$ min) [9]. Assuming unchanged cerebral blood flow, this allows for the calculation of the amount of ammonia absorbed by the tissues of the head (predominantly the brain) from the blood: 2.4, 4.5, 2.4, and 3.2 μmol per 1 g of tissue over 40 min for intact animals, those receiving fentanyl, those subjected to heat stress, or the combined action of both factors, respectively. However, these data do not reveal a consistent correlation with the intergroup differences in lethality.

The increase in glutamine content in the brain tissue did not account for the binding of all the ammonia absorbed from the blood. The remaining portion of ammonia, which crossed the blood–brain barrier over 40 min, can be determined by subtracting the increase in tissue glutamine content from the amount of ammonia absorbed by the tissue. This value amounts to 2.4, 4.3, 1.4, and 2.2 μmol per 1 g of tissue for intact animals, those receiving fentanyl, those subjected to heat stress, or the combined action of both factors, respectively. In neurons, ammonia is stoichiometrically incorporated

into glutamate and other amino acids. Since the calculated amounts of unconsumed ammonia are an order of magnitude higher than the content of pyruvic (0.06–0.13 $\mu\text{mol/g}$) and α -ketoglutaric (0.12–0.19 $\mu\text{mol/g}$) oxoacids in the nervous tissue [10], their amination to form alanine and glutamate could have reduced the availability of these key intermediates of energy metabolism for the brain tissue.

The availability of pyruvate could also have been reduced by its carboxylation to form oxaloacetate. This process is stimulated by low oxaloacetate concentration, which is linked to the concentration of α -ketoglutarate; an excess of ammonia in tissues leads to the amination of precisely these keto acids. The depleting effect of ammonia on the pool of Krebs cycle intermediates in neurons, which triggers a cascade of neuronal damage, is considered by some authors to be a primary mechanism of its neurotoxicity [11]. Another potential mechanism of neurotoxicity for ammonia not incorporated into glutamine could be the accumulation of the excitotoxic agent, glutamate, in the brain [12]. As evident from the data obtained, the intergroup differences in the calculated amounts of ammonia involved in these processes could not explain the potentiation of the acute toxicity of fentanyl under the action of heat stress.

A 2.0–2.2-fold increase in blood ammonia levels, which distinguished rats after combined exposure to fentanyl and heat stress from those subjected to isolated exposure, is generally considered non-lethal [8]. However, it can be hypothesized that in animals that died before removal from the thermal chamber, hyperammonemia was more severe than in those that did survive. Furthermore, hyperammonemia was accompanied by hypoxemia, indicated by fundal cyanosis, and metabolic acidosis, marked by lactic acidemia. The presence of both these disturbances and the maximal severity of lactic acidemia distinguished animals subjected to the combined action of fentanyl and heat stress from those subjected to their isolated effects. Hypoxia and metabolic acidosis are known to increase the toxicity of ammonia [13, 14]. Thus, it is evident that hyperammonemia, in combination with hypoxemia and lactic acidemia, reduced the survival of rats under the conditions of fentanyl and heat stress exposure.

Oxygen consumption by aerobic cells is an integral indicator of their metabolic activity [15]. Oxygen consumption by brain cells was measured in vitro under the conditions free from substrate limitations and with the ATP-dependent function of nerve impulse generation and conduction suppressed. It ranged 3.4–4.1 μL per 1 g of tissue per minute, which is an order of magnitude lower than the in-vivo rate of 33 μL per 1 g of tissue per minute [7]. The data obtained did not reveal any impairments in cellular respiration in rats that survived exposure to fentanyl and heat stress that would prevent the meeting of the energy demands of the nervous tissue under these favorable in-vitro conditions. The observed intensification of oxygen consumption by brain

homogenates from these animals was compensatory in nature and reflected a state of energy deprivation in which the brain tissue existed prior to decapitation. Given the presence of lactic acidemia and signs of hypoxemia in these animals, positive modulators of cellular respiration — ADP, AMP, and phosphate — were likely accumulating in their brain tissue [16].

Since the tissue was diluted 15-fold during homogenate preparation, the stimulatory effect of these substances was manifested only in a weakened form. Under isolated fentanyl exposure, the aforementioned disturbances might have been mitigated by a reduced demand for ATP in the brain due to the comatose state [17], which is a probable reason for the absence of significant changes in oxygen consumption by the brain homogenates. The lack of substantial changes in blood lactate levels and its arteriovenous gradient in these animals indicated an absence of significant energy debt in their brain tissue. In rats subjected to isolated heat stress, the bright scarlet color of the ocular fundus suggested intense cerebral blood flow and high oxygen saturation of arterial hemoglobin. In these animals, the lower oxygen consumption by brain cells in vitro compared to intact rats might reflect the accumulation of a negative modulator of cellular respiration — ATP.

The data obtained demonstrate that a triad of metabolic disturbances — lactic acidemia, hypoxemia, and

hyperammonemia — mediates the aggravation of acute fentanyl intoxication by heat stress in rats. While the emergence of the former two components of this triad during hyperthermia and impaired external respiration is expected, the mechanisms underlying hyperammonemia remain to be identified. In light of the cellular stress concept [18] and considering its impact on intestinal permeability [19], it is pertinent to evaluate the role of the gut barrier in the development of hyperammonemia during body hyperthermia in the setting of acute fentanyl intoxication.

CONCLUSION

1. Simulating microclimatic conditions that induce non-lethal acute body hyperthermia increased the toxicity of fentanyl in rats, raising the 40-min lethality to 68–71% compared to 0–9% at room temperature.

2. The potentiation of the lethal effect of fentanyl by heat stress in rats was not caused by irreversible thermal damage to biological tissues, organism dehydration, cerebral edema, swelling, hyperemia, or glutamine accumulation in the brain.

3. Hypoxemia, lactic acidemia, and hyperammonemia were necessary conditions for the aggravating effect of conditions promoting body hyperthermia on acute fentanyl intoxication in rats.

References

1. Ivnitsky YuYu, Vakunenkova OA, Golovko AI, Lapina NV, Rejniuk VL. The effect of general hyperthermia and local cooling on fentanyl tolerance in rats. *Extreme Medicine*. 2025;27(4):475–82 (In Russ.). <https://doi.org/10.47183/mes.2025-311>
2. Whitehead TP, Whittaker SR. A method for the determination of glutamine in cerebrospinal fluid and the results in hepatic coma. *Journal of Clinical Pathology*. 1955;8:81–4. <https://doi.org/10.1136/jcp.8.1.81>
3. Umbreit WW, Burris RH, Stauffer JF. *Manometric techniques and tissue metabolism*. Moscow: Foreign Literature Publishing House; 1951 (In Russ.).
4. Cordoba J, Gottstein J, Blei AT. Glutamine, myo-inositol, and organic osmolytes after porto-caval anastomosis in rat: implications for ammonia-induced brain edema. *Hepatology*. 1996;24(4):919–23. <https://doi.org/10.1002/hep.510240427>
5. Canfield JR, Sprague JE. Influence of carbon side chain length on the in vivo pharmacokinetic and pharmacodynamic characteristics of illicitly manufactured fentanyls. *Drug Testing and Analysis*. 2024;16(10):1113–21. <https://doi.org/10.1002/dta.3636>
6. Yarmolenko PS, Moon EJ, London 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>
7. Ivanov KP. Brain hypoxia and death of neurons as the result of disorder of microcirculation in the brain and of regional brain circulation. *Regional Blood Circulation and Microcirculation*. 2010;9(2):5–17 (In Russ.). EDN: [MUHMHV](#)
8. Kosenko EA, Kaminsky YG. *Cellular mechanisms of ammonia toxicity*. Moscow: LKI Publishing House; 2008 (In Russ.).
9. Osipov AP, Ibishov DF, Rastorgueva SL. *Physiology of blood and lymph circulation*. Perm: IPC “Prokrost”; 2022 (In Russ.).
10. Tashlitsky VN, Artiukhov AV, Fedorova NV, Sukonnikov MA, Ksenofontov AL, Bunik VI, et al. Analysis of content of 2-oxoacids in rat brain extracts using high-performance liquid chromatography. *Biochemistry (Moscow)*. 2022;87(4):356–65. <https://doi.org/10.1134/s0006297922040058>
11. 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>
12. Ommati M, Mobasheri A, Hossein N, Rezaei M, Alidaee S, Arjmand A, et al. Low-dose ketamine improves animals' locomotor activity and decreases brain oxidative stress and inflammation in ammonia-induced neurotoxicity. *Journal of Biochemical and Molecular Toxicology*. 2023;37(11):e23468. <https://doi.org/10.1002/jbt.23468>
13. Kenneth S. Hypoxia and ammonia toxicity. *American Journal of Physiology*. 1960;199:1105–8. <https://doi.org/10.1152/ajplegacy.1960.199.6.1105>
14. Podosynovickova NP, Schäfer TV, Rejniuk VL, Ivnitsky JuJu. Effect of hypoxia on sodium and ammonium acetate toxicity for Daphnia. *Bulletin of Experimental Biology and Medicine*. 2010;149(6):712–3. <https://doi.org/10.1007/s10517-010-1032-y>
15. Pamerter M, Dzal Y, Thompson W, Milsom W. Do naked mole rats accumulate a metabolic acidosis or an oxygen debt in severe hypoxia? *Journal of Experimental Biology*. 2019;222(Pt 3):jeb191197.

16. Semenov DG, Belyakov AV, Rybnikova EA. Experimental modeling of damaging and protective hypoxia of the mammalian brain. *Journal of Evolutionary Biochemistry and Physiology*. 2022;58:2021–34.
<https://doi.org/10.1134/S0022093022060291>
17. Judenherc-Haouzi A, Lewis T, Reinhardt A, Haouzi P. Life-threatening fentanyl overdose beyond medullary depression in breathing. *American Journal of Physiology*. 2025;328(3):R408–21.
<https://doi.org/10.1152/ajpregu.00238.2024>
18. Lu S, Wei F, Li G. The evolution of the concept of stress and the framework of the stress system. *Cell Stress*. 2021;5(6):76–85.
<https://doi.org/10.15698/cst2021.06.250>
19. Lee BJ, Russell SL, Meade RD, McCormick JJ, King KE, Kenny GP. Markers of enterocyte damage, microbial translocation, and systemic inflammation following 9 h of heat exposure in young and older adults. *Applied Physiology, Nutrition and Metabolism*. 2024;49(9):1241–51.
<https://doi.org/10.1139/apnm-2024-0094>

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PROSPECTS FOR APPLYING EYE TRACKING IN FUNCTIONAL STATE ASSESSMENT OF PILOTS

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Introduction. The development and implementation of improved methods for assessing the functional state of pilots during their professional activities remains a priority direction in aviation medicine due to the need to preserve the professional health and reliability of pilots and, consequently, ensure flight safety. According to contemporary research, eye tracking is increasingly gaining application in medical and paramedical fields. Human oculomotor activity is influenced by numerous internal and external factors, especially during the performance of various cognitive tasks. Therefore, its assessment holds significant potential for diagnosing the functional state of individuals in extreme professional settings.

Objective. To study and substantiate the potential of applying eye tracking technologies for assessment of the functional state of pilots.

Materials and methods. The study involved 22 pilots who were requested to perform flights on aviation simulators with simulated emergency and abnormal situations. The pilots were divided into two groups based on the correctness of their actions in emergency situations. Group 1 ($n = 10$) (mean age 25.5 ± 2 years) was rated in the range of 7–8 points on a 10-point scale, while Group 2 ($n = 12$) (mean age 31.5 ± 2 years) was rated in the range of 8–9 points. The functional state of the pilots was assessed both before and after the simulator flight during the performance of various cognitive tasks. Parameters of heart rate regulation (via mathematical-statistical and spectral parameters of the cardiogram), balance and mobility of nervous processes (assessment of simple and complex sensorimotor reactions), attention properties, and oculomotor activity using an eye tracker were recorded. Statistical data processing was performed using the Microsoft® Excel-2016 software and the SPSS 26 application software package.

Results. In Group 1, the pilots demonstrated a decrease in saccadic velocity characteristics, signs of strain of the body's adaptation mechanisms according to heart rate variability data, and a reduction in the functional level of the central nervous system. In Group 2, the pilots showed no significant changes in fixation and gaze movement characteristics. However, the cardiogram revealed moderate strain of adaptation mechanisms and an increased functional level of the central nervous system. Correlation analysis of the data obtained established tight statistical relationships of the velocity parameters of gaze movement with both heart rate variability parameters (PARS, $r_s = 0.53$; $p \leq 0.01$) and attention properties, such as capacity ($r = -0.62$, $p \leq 0.01$) and distribution ($r_s = -0.57$; $p \leq 0.01$).

Conclusions. Changes in the oculomotor activity parameters of a pilot during the performance of cognitive tasks while simulating emergency and abnormal situations on an aviation simulator exhibit a co-directional pattern with the dynamics of heart rate regulation, attention properties, as well as the balance and mobility of nervous processes. Saccadic velocity parameters showed tight statistical relationships with both the integral PARS indicator, characterizing the degree of strain of the body's adaptation mechanisms, and attention capacity and distribution. Eye tracking holds significant potential for diagnosing the functional state of pilots during professional activities as a modern, effective, and integral method.

Keywords: pilot; aviation medicine; functional state; heart rate variability; central nervous system; oculomotor activity; emergency situation

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ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ АЙТРЕКИНГА В ДИАГНОСТИКЕ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ЛЕТЧИКА

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Введение. Разработка и внедрение в практику авиационного врача новых и эффективных методов оценки функционального состояния летчика в процессе его профессиональной деятельности остается одной из актуальных задач. Это обусловлено необходимостью сохранения профессионального здоровья летчика, а следовательно, профессиональной надежности и безопасности полетов. По данным современных исследователей, айттрекинг получил большое распространение в медицине и парамедицинских областях. Окуломоторная активность человека находится в зависимости от воздействия множества как внешних, так и внутренних факторов, особенно в период выполнения различных видов когнитивных задач, и имеет большой потенциал в диагностике функционального состояния людей экстремальных профессий.

Цель исследования. Изучить и научно обосновать возможности применения айттрекинга в диагностике функционального состояния летчика.

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Материалы и методы. В исследовании приняли участие 22 летчика, которые выполняли полеты на авиационных тренажерах с моделированием аварийных и нештатных ситуаций. Летчики были разделены на две группы в зависимости от правильности действий в аварийной ситуации. Группа 1 ($n = 10$) (средний возраст $25,5 \pm 2,0$ года) по 10-балльной шкале оценивалась в диапазоне от 7 до 8 баллов, а группа 2 ($n = 12$) (средний возраст $31,5 \pm 2,0$ года) — в диапазоне от 8 до 9 баллов. Функциональное состояние летчиков оценивали до и после полета на тренажере в процессе выполнения когнитивных задач. При этом регистрировали показатели регуляции сердечного ритма (посредством математико-статистических и спектральных показателей кардиоритмограммы), сбалансированности и подвижности нервных процессов (оценка простой и сложной сенсомоторных реакций), свойств внимания, а также окулomotorной активности с использованием айтрекера. Статистическую обработку данных проводили с использованием программного обеспечения Microsoft® Excel-2016, пакета прикладных программ SPSS 26.

Результаты. У летчиков группы 1 зарегистрировано снижение скоростных характеристик саккад, признаки перенапряжения адаптационных механизмов организма по данным вариабельности сердечного ритма и снижение уровня функционирования центральной нервной системы. В группе 2 у испытуемых не зафиксировано достоверных изменений характеристик фиксации и перемещения взора, при этом при анализе кардиоритмограммы зафиксировано умеренное напряжение адаптационных механизмов, а также повышение уровня функционирования центральной нервной системы. При корреляционном анализе полученных данных установлены сильные статистические связи скоростных показателей перемещения взора с показателями вариабельности ритма сердца (ПАРС, $r_s = 0,53$; $p \leq 0,01$) и свойствами внимания, такими как объем ($r = -0,62$, $p \leq 0,01$) и распределение ($r_s = -0,57$; $p \leq 0,01$).

Выводы. Изменение показателей окулomotorной активности летчика при выполнении когнитивных задач в процессе моделирования аварийных и нештатных ситуаций на авиационном тренажере имеет сопоставленный характер с динамикой регуляции сердечного ритма, свойств внимания, баланса и подвижности нервных процессов. Скоростные показатели саккад имеют сильные статистические связи с интегральным показателем ПАРС, характеризующим степень напряжения адаптационных механизмов организма, а также с объемом и распределением внимания. Айтрекинг имеет большой потенциал в диагностике функционального состояния летчика в процессе профессиональной деятельности как современный эффективный интегральный метод.

Ключевые слова: летчик; авиационная медицина; функциональное состояние; вариабельность сердечного ритма; ЦНС; окулomotorная активность; аварийная ситуация

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Финансирование: исследование выполнено без спонсорской поддержки.

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

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INTRODUCTION

According to research [1, 2], measures aimed at preserving pilot health and predicting its dynamics are essential elements of modern aviation flight safety. In this regard, functional state assessment of pilots and their performance during professional activity have become routine and mandatory tasks of aviation physicians.

Health-assessment activities are conducted not only directly during flight operations but also on aviation simulators and when conducting psychophysiological training of pilots for the effects of various flight factors [3–5]. The dynamics of the functional state of a pilot's body is monitored during application of special research methods, medical examination of flight personnel by medical boards [6], training of cadets at flight training organization [7], as well as when simulating the influence of extreme aviation flight factors in ground-based conditions [7, 8].

At present, there exist a substantial number of methods for assessing the functional state and professional performance of pilots. Russian researchers have developed both monoparametric approaches, where physiological, psychophysiological, and psychological

indicators of an individual are recorded and comprehensively evaluated, as well as multiparametric solutions. For example, a large body of work is dedicated to assessing heart rate regulation as an integral indicator of a pilot's functional state, with the possibilities of mathematical and spectral analysis of heart rate variability being described in detail [8, 9]. Research into the potential of computerized stabilometry for evaluating vestibular function as a statokinetic functional system in humans is underway [10]. In addition, numerous other techniques and approaches have been studied and developed with the purpose of proposing integrated, valid, and highly convenient practical solutions for aviation physicians to monitor the functional state of pilots.

Eye tracking is a highly promising technology for assessing the functional state of pilots based on oculomotor activity. This technology has been studied for several decades [11], finding wide application in medicine, e.g., in neurophysiology, neurosurgery, psychiatry, and ophthalmology [12, 13], as well as in a number of paramedical fields [14]. It should be noted that the capabilities of eye tracking in aviation medicine have already been addressed; however, these works primarily focused on the issues of information perception

and attention distribution, which are key for aviation ergonomics. For instance, assessment of the functional state and performance of civil aviation pilots [15, 16] and cadets during flights on aviation simulators was undertaken.¹ A distinctive feature of this work was the evaluation of a pilot's oculomotor activity during a flight on an aviation simulator. According to prominent Russian researchers, human oculomotor activity is directly linked to the process of solving cognitive tasks, with the efficiency of their solution being strongly affected by various internal and external factors [12]. Thus, eye tracking holds considerable potential for assessing the impact of various factors associated with the professional activity of pilots on their functional state and performance.

Considering the above, the psychophysiological foundations for using oculomotor activity indicators in diagnosing the functional state of pilots during their professional activities are of great interest. In an attempt to address this problem, we conducted a study to assess the functional state of pilots during ground-based training on aviation simulators. The aim was to evaluate the possibility of using eye tracking for assessing the functional state of pilots.

MATERIALS AND METHODS

The study involved 22 male pilots from Southeast Asia aged 24–47 years (mean age 28.8 ± 2 years). At the time of examination, the participants had no acute conditions or exacerbations of chronic diseases, including those affecting the visual and nervous systems. All pilots have had practical experience in real flights and simulator training with simulated emergency situations in flights on Yak-130 aircrafts.

During the study, the pilots underwent training on Yak-130 aviation simulators. Each pilot performed one flight according to a predetermined route and flight parameters. During the flight, the flight director simulated the impact of an external stress factor by creating an abnormal emergency situation, such as failures of control surfaces and systems, engine failures, deployment of the drag chute in flight, etc. Only one abnormal situation was simulated per flight. In the emergency situation, the pilot had to perform a series of actions according to the algorithm and complete the flight. Upon completion of the task, the flight director evaluated the pilot's actions on a 10-point scale in accordance with the protocol established by the governing documents.

The participants were divided into two groups based on the results of the simulator flight (Yak-130, Russia).

Group 1 included 10 pilots with an average score of 7.3 within the range of 7–8 points. The mean age in this group was 25.5 ± 2 years, and the mean total flight time was 261 h. Group 2 consisted of 12 pilots who received

an average score of 8.2 within the range of 8–9 points. Their mean age was 31.5 ± 2 years, and their flight time was 603 h. Thus, the formed groups differed in terms of not only simulator training results but also significant differences in age and professional skills. The latter factor was likely to affect the former factor.

In order to assess the dynamics of the functional state of pilots under the influence of a stress factor, they were examined 15 min before performing the simulator flight and within 15 min immediately after completing the flight. The functional state of pilots during the pre-flight period and after the simulator flight was diagnosed according to a unified plan. This plan involved assessment of heart rate regulation (HRR) and the functional level of the central nervous system (CNS), as well as recording oculomotor activity in the pilots while performing a cognitive task.

HRR was assessed through the analysis of statistical indicators of the cardiogram: mode (Mo, ms), reflecting the functional level of the sinus node; amplitude of mode (AMo, %), indicating the activity of the sympathetic nervous system; variation range (VR, ms), associated with the activity of the parasympathetic division of the autonomic nervous system; stress index of regulatory systems (SI, rel. units), which reflects the activity of sympathetic rhythm regulation. Spectral indicators included power of high-frequency oscillations (HF, ms^2), reflecting parasympathetic influence on the rhythm; low-frequency oscillations (LF, ms^2), associated with sympathetic nervous system activity; very low-frequency oscillations (VLF, ms^2), associated with humoral influence on heart rhythm; the LF/HF ratio (rel. units), indicating the balance of sympathetic and parasympathetic nervous system influences on the rhythm; total power of the spectrum (TP, ms^2), reflecting the total autonomic influence on heart rhythm; and the integral indicator of regulatory system activity (PARS, rel. units) [17], recorded over 5 min using the VNS-Ritm equipment (Russia).

The CNS state was investigated using the NS-Psikhotest equipment (Russia) by recording simple and complex sensorimotor reactions (SSR and CSR), reaction to a moving object (RMO), critical flicker fusion frequency (CFFF). The stability and concentration of attention were assessed by comparing the results obtained by the Noise Immunity and Attention Assessment methods. During the Schulte–Platonov Black-Red Tables test, attention capacity (AC), distribution (AD), and switching (AS) were determined on a five-point scale. Here, attention capacity was assessed across the ranges: 0–29, 30–37, 38–50, 51–60, 61 and above points. Attention distribution was assessed as follows: 0–43, 44–56, 57–87, 88–106, 107 and above points. Attention switching was determined across the ranges: 0–9, 10–17, 18–31, 32–40, 41 and above points.²

¹ Zibarev EV. Scientific substantiation of the concept for assessing work intensity in civil aviation pilots: dissertation for the degree of Doctor of Medical Sciences. 2023.

² Mantrova IN. Methodological guide to psychophysiological and psychological diagnostics. Ivanovo; 2005.

Recording of oculomotor activity was conducted using an eye tracker (SMI RED250, Germany) while the pilot performing a cognitive task in the form of the Schulte–Platonov Black-and-Red Tables test. Analysis of the recorded data was performed using the standard SMI BeGaze software for this equipment. Quantitative indicators of fixations and saccades (number and frequency) were analyzed; temporal parameters included duration of fixations and saccades (short — < 200 ms, medium — 200–350 ms, and long — > 350 ms fixations); saccadic velocity characteristics included peak velocity, average velocity, and amplitude [12].

Statistical analysis was performed using the Microsoft® Excel-2016 software, the SPSS 26 application software package, with calculation of the Wilcoxon test. The values of all indicators are presented as the median (M_e) with the upper and lower quartiles [Q_1 ; Q_3], corresponding to the 25th and 75th percentiles of the distribution. The indicators recorded before and after the simulator flight were compared. All differences were considered statistically significant at a significance level of $p < 0.05$. Correlation analysis was performed using Spearman's rank correlation coefficient, with the indicators being considered statistically significant at a level of $p < 0.05$.

The pilots were examined in the morning in a quiet, well-ventilated separate room at an air temperature of +22°C. All participants provided voluntary consent to participate in the study, which was conducted in accordance with biomedical ethics standards (Declaration of Helsinki and European Community directives, 8/609 EC).

RESULTS

When investigating oculomotor activity (quantitative and temporal characteristics of fixations) in pilots before and after simulating an emergency situation on an aviation simulator, no statistically significant changes were

detected in either group, except for a 9% decrease ($p < 0.05$) in the number of fixations in Group 2. The corresponding data are presented in Table 1.

The results of assessing gaze movement characteristics — quantitative, temporal, and velocity parameters of saccades — are reflected in Table 2. Statistically significant changes were registered in Group 1. These were presented as a decrease in saccadic velocity parameters: a 9% decrease in peak saccadic velocity ($p < 0.05$), a 6% decrease in average velocity ($p < 0.05$), and a 17% decrease in saccadic amplitude ($p < 0.05$), while quantitative and temporal characteristics showed no significant changes. In Group 2, significant changes were noted only in an 8% decrease in the number of saccades ($p < 0.05$).

In order to assess the dynamics of the functional state of pilots during simulation of an in-flight emergency and to provide a physiological rationale for the nature of changes in oculomotor activity indicators, an assessment of HRR was conducted.

In Group 1, the following statistically significant changes were recorded: an 18% increase in the VR indicator (from 193 to 227 ms), a 12% increase in TP (from 3756 to 4194 ms²), a 46% increase in VLF (from 1200 to 1780 ms²), a 25% increase in the PARS indicator (from 3.0 to 3.75 points), and a 26% decrease in HF (from 1629 to 1250 ms²), and a 16% decrease in SI (from 155 to 131 rel. units) (Fig. 1).

In Group 2, statistically significant changes were also noted: an increase in AMo by 32% and SI by 67% (from 293 to 488 rel. units); a decrease in TP by 33% (from 2602 to 1749 ms²), LF by 39% (from 1153 to 699 ms²), and the LF/HF ratio by 30% (from 1.93 to 1.35 rel. units) (Fig. 2).

The impact of the pilot's actions in an emergency situation during simulated flight conditions on an aviation simulator on their functional state was assessed by changes in the functioning level of the CNS. To that end, the rate of visual-motor reactions, the mobility of

Table 1. Characteristics of fixations in pilots before and after simulating an emergency situation on an aviation simulator

Parameter	Pilot group	Baseline value	Value after the simulator flight
Number of fixations, units	Group 1	240 [191; 257]	232 [222; 256]
	Group 2	348 [259; 338]	315 [276; 308]*
Fixation frequency, units per sec	Group 1	7.33 [7.04; 8.00]	7.53 [7.08; 8.21]
	Group 2	7.36 [6.91; 7.94]	7.45 [7.05; 8.04]
Fixation duration, ms	Group 1	246 [217; 261]	244 [220; 254]
	Group 2	232 [214; 254]	232 [219; 246]

Table compiled by the authors based on their own data

Note: * — level of statistical significance $p \leq 0.05$ when comparing indicators before and after the simulator flight; data are presented as the median (M_e) with upper and lower quartiles [Q_1 ; Q_3].

Table 2. Characteristics of saccades in pilots before and after simulating an emergency situation on an aviation simulator

Parameter	Pilot group	Baseline value	Value after the simulator flight
Number of saccades, units	Group 1	235 [181; 255]	228 [218; 254]
	Group 2	337 [249; 336]	309 [270; 301]*
Saccade frequency, units per sec	Group 1	7.10 [6.51; 7.90]	7.41 [6.93; 8.13]
	Group 2	7.14 [6.81; 7.90]	7.32 [6.86; 7.94]
Saccade duration, ms	Group 1	23.7 [21.940; 23.955]	23.3 [21.8; 24.4]
	Group 2	26.9 [25.1; 29.1]	26.8 [24.7; 29.0]
Saccade amplitude, degrees	Group 1	1.86 [1.38; 1.78]	1.53 [1.29; 1.62]*
	Group 2	1.93 [1.51; 1.94]	1.91 [1.52; 1.90]
Peak saccade velocity, degrees per sec	Group 1	199 [166; 220]	181 [155; 204]*
	Group 2	199 [168; 231]	189 [160; 198]
Average saccade velocity, degrees per sec	Group 1	65.9 [56.1; 71.5]	62 [53; 69]*
	Group 2	63.5 [54.6; 70.1]	63 [55; 67]

Table compiled by the authors based on their own data

Note: * — level of statistical significance $p \leq 0.05$ when comparing indicators before and after the simulator flight; data are presented as the median (M_e) with upper and lower quartiles [Q_1 ; Q_3].

nervous processes in the visual analyzer, the balance of excitation and inhibition processes, as well as attention characteristics were recorded.

In Group 1, pilots exhibited significant changes in a number of indicators: a 37% decrease in the absolute

negative value of RMO time (from -12.7 to -8.0 ms), a 50% increase in attention distribution indicators (from 71.9 to 108.0 points), and a 94% increase in attention switching indicators (from 39.4 to 76.3 points) compared to baseline values ($p < 0.05$). Other psychophysiological

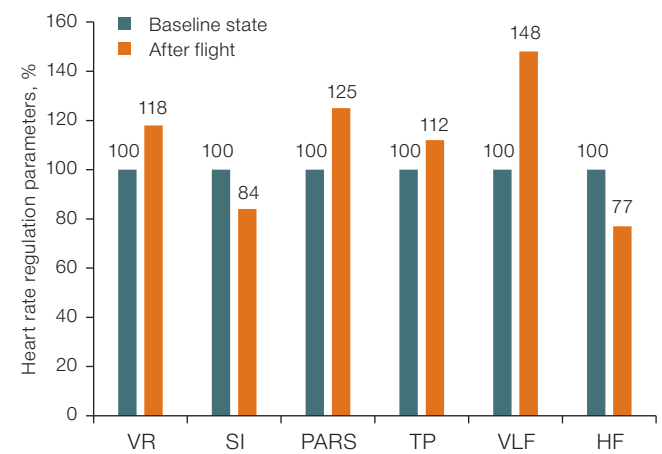


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Fig. 1. Results of statistical and spectral analysis of cardiorythmograms in Group 1 before and after a simulation flight: VR — variation range, SI — stress index of regulatory systems, PARS — indicator of regulatory system activity, TP — total power of the spectrum, VLF — power of very low frequency oscillations, HF — power of high frequency oscillations

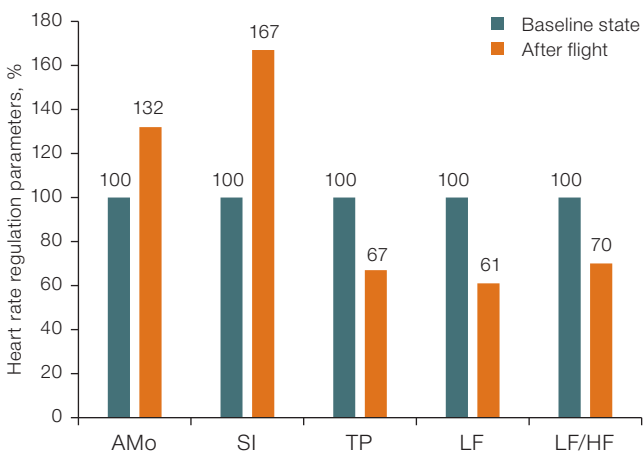


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Fig. 2. Results of statistical and spectral analysis of cardiorythmograms in Group 2 before and after a simulation flight: AMo — amplitude of mode; SI — stress index of regulatory systems; TP — total power of the spectrum; LF — power of low frequency oscillations; LF/HF — ratio of low to high frequency oscillation power

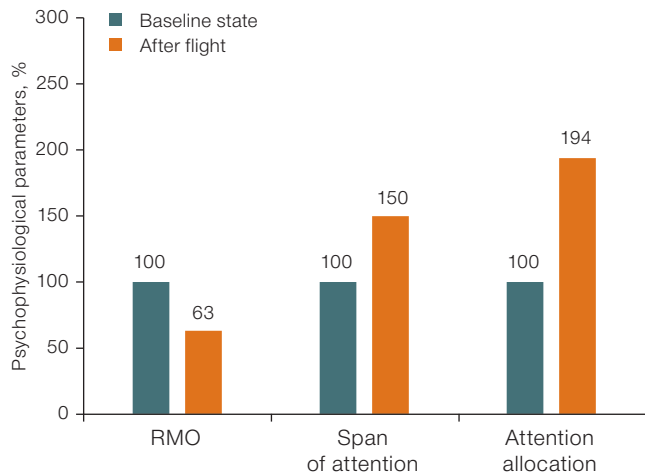


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Fig. 3. Dynamics of reaction to a moving object and attention characteristics in pilots of Group 1 before and after a simulation flight: RMO — reaction to a moving object

indicators, including attention stability and concentration, showed no statistically significant changes (Fig. 3).

When assessing sensorimotor reactions in Group 2, the pilots also showed significant changes: a 2.2-fold increase in the absolute negative value of RMO from -6.1 to -13.0 ms ($p < 0.05$) and a 2.6-fold increase in attention switching from 6.8 to 17.7 points ($p < 0.05$). Other psychophysiological indicators, similar to those in Group 1, showed no statistically significant changes (Fig. 4).

In order to identify statistical relationships between oculomotor activity, heart rate regulation characteristics, and the CNS functioning level during cognitive task performance, a correlation analysis was performed. The data obtained before, after the start, and immediately

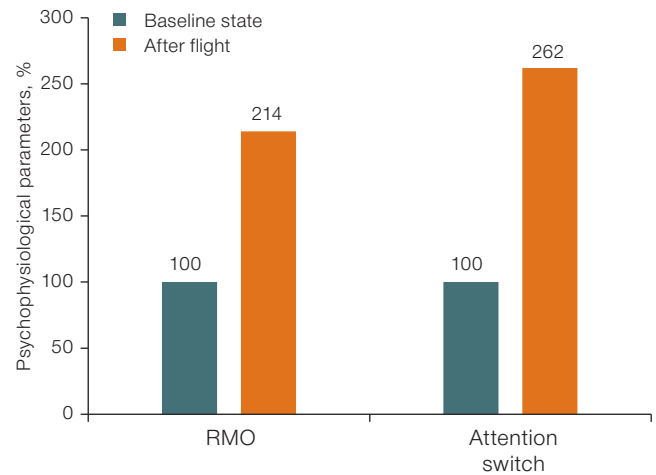


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Fig. 4. Dynamics of reaction to a moving object and attention characteristics in pilots of Group 2 before and after a simulation flight: RMO — reaction to a moving object

upon the completion of the simulation flight were investigated. The most significant results of the correlation analysis are presented in Table 3.

Tight inverse correlations between velocity indicators of oculomotor activity and attention capacity and distribution were established, along with tight direct correlations with the indicator of regulatory system activity and the balance ratio of low- to high-frequency oscillation power.

DISCUSSION

The analysis of gaze stability based on fixation characteristics, which revealed no statistically significant changes, indicates the absence of a target for these

Table 3. Correlation analysis of heart rate regulation parameters, CNS functioning level, and oculomotor activity in pilots before and after simulation flights, ($n = 22$)

Parameter	Before the simulation flight			After the simulation flight		
	Span of attention	Attention allocation	PARS	Span of attention	LF/HF	PARS
Proportion of short fixations	—	—	—	—	0.44*	0.69*
Proportion of medium fixations	—	—	—	—	0.42*	0.54*
Saccade duration	-0.74^*	-0.60^*	—	-0.83^*	—	0.64^*
Saccade amplitude	-0.43^*	-0.52^*	0.45^*	-0.53^*	—	0.53^*
Peak saccade velocity	-0.56^*	-0.57^*	0.48^*	-0.62^*	—	0.51^*
Average saccade velocity	-0.61^*	-0.55^*	0.44^*	-0.66^*	—	0.48^*

Table compiled by the authors based on their own data

Note: statistical significance level * — $p \leq 0.05$; * — $p \leq 0.01$; * — $p \leq 0.001$; LF/HF — low to high frequency oscillation power ratio, PARS — integral indicator of regulatory system activity.

oculography parameters in the present study. The number and duration of fixations largely reflect the visual analyzer state when solving complex cognitive tasks or under challenging conditions for their execution [12–14]. In the conducted study, cognitive tasks were solved before and after simulating an emergency situation, rather than during the performance of the flight task on the simulator, when the pilot is exposed to the maximum spectrum of professional stress factors. Consequently, various parameters of gaze fixation did not show significant changes.

At the same time, the assessment of gaze movement results revealed a significant decrease in the velocity parameters of saccades in pilots with lower performance results on the simulator (Group 1). According to a number of authors, saccades exert a certain reciprocal influence on cognitive function, and a decrease in saccadic velocity is observed in neurophysiological disorders [12]. In the presented study, the presence of diseases or pathological conditions in the pilots was excluded at the stage of forming the subject group. Therefore, the described features of gaze movement may be due to changes in the functional state of Group 1 pilots under the influence of stress factors during the simulation of an emergency situation, which inevitably exerts autonomic influence on the functioning of all human analyzers, including the visual ones. The most likely functional state in Group 1 with lower simulator flight results after the flights is fatigue. This state, although being characterized as normal, is accompanied by the onset of a decline in human performance and the functional level of physiological systems.

The analysis of HRR as an integral indicator of the functional state of pilot health allowed us to draw several conclusions. Pilots in Group 1 showed pronounced tension in the regulatory systems with a tendency towards strain of the adaptation mechanisms. In a study by Baevsky et al., strain was characterized by somewhat discordant changes in HRR indicators [8]. On the one hand, Group 1 showed a decrease in the index of tension of regulatory systems and an increase in the variation range, reflecting increased parasympathetic influence. On the other hand, there was an increase in total spectral power, indicating an overall reduction in autonomic influence and centralization of rhythm control. However, this reduction is primarily due to a decrease in parasympathetic influence, which somewhat contradicts the data from variational pulse oximetry. The increase in the PARS indicator reflects a shift in the tension of regulatory systems into the area of pronounced tension [8, 17].

From a physiological standpoint, concordant changes in heart rate variability parameters and saccadic velocity indicators can be observed, reflecting a decrease in cognitive activity and likely fatigue of the visual analyzer.

The assessment of HRR in Group 2 before and after simulating emergency situations on a simulator indicated

an increase in the centralization of rhythm control and overall autonomic influence thereon. This is confirmed by a significant increase in SI, sympathetic influence (AMo), and a decrease in total spectral power. The decrease in TP is due to a statistically significant reduction in sympathetic influence; however, a trend toward a synchronous decrease in parasympathetic and humoral influence can be observed [8].

The above HRR results, considering the absence of significant changes in fixation and saccade parameters during oculography, allow us to conclude that pilots in Group 2 developed a normal functional state with moderate load on adaptation mechanisms and body regulatory systems, while maintaining professional performance when performing simulation flights.

The results obtained when studying the CNS functioning level and attention characteristics in pilots before and after simulating emergency situations on a simulator demonstrated different baseline values in the two groups. In Group 1, the pilots (with lower simulator flight results) exhibited a significant predominance of excitation over inhibition processes, unlike the pilots in Group 2. The same situation was observed when considering attention characteristics. Group 1 registered indicators corresponding to low mobility of nervous processes, while in Group 2 its higher mobility was noted. The attention characteristics fully confirm the difference in the baseline functional state of the pilots, as described by the reaction to a moving object. These differences can be explained by the varying level of professional skills of the pilots in the two groups and their level of psychological readiness to act in conditions of emergency and abnormal flight situations.

The dynamics of these parameters, recorded immediately after the flight in Group 1, reflects an even greater decrease in the functioning level of the CNS compared to baseline. This can be interpreted as strain of the body's adaptation mechanisms [17]. This fully corresponds to the results obtained from oculography and the analysis of saccadic velocity indicators in Group 1, which indicate a decrease in cognitive activity and, most likely, the development of fatigue.

In Group 2, after flights on a simulator, an increased predominance of excitation processes in the CNS was noted, which can be characterized as the mobilization of psychophysiological reserves. The absence of significant changes in attention characteristics, except for a slight decrease in switching capacity (from level 5 to 4), cannot indicate a significant reduction in the mobility of nervous processes.

These results, considering the absence of significant changes in saccadic velocity indicators and gaze fixation characteristics in Group 2 pilots, indicate the preservation of their normal functional state while maintaining their professional performance, which is ensured by moderate tension in the regulatory systems [17].

The conducted correlation analysis of oculomotor activity parameters with HRR indicators and CNS

functioning level indicated a close statistical relationship of the velocity indicators of oculomotor activity with the PARS integral heart rate variability indicator. An increase in its values reflects tension in the body's regulatory mechanisms. Inverse correlations were established with attention properties, such as capacity and distribution, which reflects a connection between the activation of oculomotor activity and the complexity of cognitive tasks, as well as the mobilization of psychophysiological reserves. To a lesser extent, saccadic velocity parameters were associated with indicators of the spectral analysis of rhythmograms and the CNS functioning level.

CONCLUSIONS

The results obtained when assessing the oculomotor activity of pilots during cognitive task performance under simulated emergency conditions on an aviation simulator allow us to draw the following conclusions:

1. The analysis of oculomotor activity in conjunction with heart rate regulation parameters, CNS functioning level, and attention characteristics indicated the development of fatigue in pilots with lower simulator flight results. At the same time, pilots from Group 2 with a higher level of psychophysiological reserves demonstrated preservation of a normal functional state. Oculomotor activity indicators show statistically significant changes against

the background of functional state dynamics during professional activity.

2. The dynamics of velocity indicators of oculomotor activity in pilots, such as saccadic velocity and amplitude, are co-directional with changes in heart rate regulation. This reflects a certain degree of load on the body's regulatory systems and the mobilization of physiological reserves. This is demonstrated by the dynamics of the index of regulatory systems tension, the indicator of regulatory system activity, as well as the spectral parameters of the cardiorythmogram.

3. Changes in oculomotor activity indicators are co-directional with the dynamics of attention capacity and distribution, the balance and mobility of nervous processes. This reflects the functioning level of the central nervous system.

Velocity indicators of oculomotor activity have a tight, statistically significant correlation with the PARS integral heart rate regulation indicator, as well as with attention capacity and distribution. This indicates their dependence on the body's overall functional state. Analysis of oculomotor activity indicators allows the functional state of pilots to be assessed. In order to fully implement the potential of eye tracking technologies for assessment of the functional state of pilots, it seems advisable to evaluate the dynamics of oculomotor activity indicators during the direct impact of professional activity factors, as well as during analytical mental work.

References

1. Ponomarenko VA, Alekseenko MS, Dolgov AA. Psychophysiological components of professional reliability of pilot. *Flight Safety Issues*. 2018;6:3–18 (In Russ.). EDN: [QTYBBP](#)
2. Zhdanko IM, Isaenkov VE, Vorona AA, Filatov VN, Nikiforov DA. Professional reliability of military pilots: medical and social-psychological aspects. *Military Medical Journal*. 2016;337(6):30–6 (In Russ.). EDN: [WLBOWT](#)
3. Blaginin AA, Lapshina TA, Dang QH. The efficiency of different training regimes at statoergometer to increase the tolerance to aerobatic overloads in vietnamese men. *Kremlin Medicine Journal*. 2022;4:32–5 (In Russ.). <https://doi.org/10.48612/cgma/z5xh-auvp-pgv4>
4. Blaginin AA, Lapshina TA, Emelyanov YuA, Bacovets DV, Dudina EA. Moderate hypoxia tolerance by military females in different phases of their ovarian-menstrual cycle. *Kremlin Medicine Journal*. 2024;2:64–7 (In Russ.). <https://doi.org/10.48612/cgma/4b2h-5829-gr7x>
5. Lapshina TA, Blaginin AA, Emelyanov YuA, Bacovets DV. Assessment of static muscular endurance of the lower extremities in female military personnel. *Professional health of military personnel*. Materials of the All-Army scientific and practical conference (on the 100th anniversary of the birth of Professor I.D. Kudrin). St. Petersburg; 2023 (In Russ.). EDN: [GMFYLR](#)
6. Sergoventsev AA, Pastukhov AG, Zemlyakov SV, Datsko AV, Churilov YuK, Vovkodav VS, et al. Development of the system of medical examination of aviation personnel of the state aviation of the Russian Federation. *Military Medical Journal*. 2021;342(3):66–73 (In Russ.). EDN: [ZUQWCV](#)
7. Barinov SV, Korsunov SV. The main content of theoretical fundamental training and practical flight training of cadets at the Higher Military Aviation School of pilots. *IX International Scientific and Practical Conference of Young Scientists dedicated to the 58th anniversary of Yuri Gagarin's flight into space*. Conference proceedings of the scientific-practical conference. Krasnodar; 2019 (In Russ.). EDN: [ABFDGE](#)
8. Baevsky RM, Chernikova AT. The problem of physiological norm: a mathematical model of functional states based on the analysis of heart rate variability. *Aerospace and Environmental Medicine*. 2002;36(6):11–7 (In Russ.). EDN: [SAAWAJ](#)
9. Annenkov OA, Ovchinnikov DV, Ivakov YuM, Sinelnikov SN, Bakovets DV. Assessment of adaptive capabilities of pilots during flight training and performance: a retrospective study. *Marine Medicine*. 2025;11(2):135–43 (In Russ.). EDN: [RUUSTQ](#)
10. Zhiltsova II, Alzhev NV. Experience of using computer posturography for pre-flight and post-flight control of the functional state of the body of pilots. *Military Medical Journal*. 2020;341(12):47–54 (In Russ.). EDN: [GDTNCT](#)
11. Zhiltsova II, Alzhev NV, Annenkov OA, Lapshina TA. Influence of psychoemotional stress on postural stability on the parameters of the statokinesiogram spectrum and heart rate variability. *Military Medical Journal*. 2018;339(6):61–9 (In Russ.). EDN: [XYPOBN](#)

12. Barabanshchikov VA, Zhegalo AV. *Eye-tracking: methods of eye movement registration in psychological research and practice*. Moscow: Kogito-Center Publ. House; 2014 (In Russ.). EDN: [TQWJHL](#)
13. Shelepin EYu, Skuratova KA, Lekhnitskaya PA, Shelepin KYu. Eye tracking as a tool for medical diagnosis. *Russian Psychological Journal*. 2024;21(4):168–94 (In Russ.). <https://doi.org/10.21702/2yd85727>
14. Fox SE, Faulkner-Jones BE. Eye-Tracking in the Study of Visual Expertise: Methodology and Approaches in Medicine. *Frontline Learning Research*. 2017;5(3):29–40. <https://doi.org/10.14786/flr.v5i3.258>
15. Taleeva AI, Zvyagina NV. Oculomotor activity in solving visual cognitive tasks under different time conditions. *Sensory Systems*. 2021;35(3):217–27 (In Russ.). <https://doi.org/10.31857/S0235009221030045>
16. Merkulova AG, Kalinina SA. The distribution of the visual attention in the training of student-pilots for the flight activity. *Hygiene and Sanitation*. 2017;96(8):752–5 (In Russ.). <https://doi.org/10.18821/0016-9900-2017-96-8-752-755>
17. Mikhailov VM. *Heart rate variability. Practical application experience*. Ivanovo: Ivanovo State Medical University' Publishing House; 2000 (In Russ.). EDN: [UBBQTR](#)

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INTERRELATIONSHIP OF NT-proBNP AND ST2 CARDIAC BIOMARKERS WITH CARDIOVASCULAR FUNCTIONAL PARAMETERS UNDER CONDITIONS OF HEAD-DOWN BED REST

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Introduction. Insufficient physical activity is a key risk factors in the development of cardiovascular diseases. Such a condition can be simulated by head-down tilt bed rest (HDBR) studies, which enable the investigation of pathophysiological changes in the heart. Previous HDBR experiments have identified structural alterations in the heart, including decreased arterial pressure, impaired autonomic regulation, and vascular remodeling. However, the molecular mechanisms underlying these processes remain poorly understood.

Objective. To investigate links between functional parameters of the cardiovascular system and NT-proBNP and ST2 cardiac biomarkers.

Materials and methods. The experiment involved six volunteers who were subjected to 21-day HDBR. The parameters of heart rate variability (HRV), dispersion mapping (ECG DM), as well as levels of NT-proBNP and ST2 protein concentrations in venous blood samples, were evaluated. The examination was conducted five days before the onset of HDBR, on Days 1, 10, and 20 during HDBR, and on Day 5 after the experimental exposure. All computations were performed using the Statistica 12 statistical software package.

Results. The NT-proBNP protein concentration significantly decreased on Days 10 and 20 of the experiment, while the ST2 protein concentration was permanently decreasing from the first days of HDBR. The left ventricular depolarization index increased sharply in the middle of the study, although having returned to their baseline values by Day 5 after the cessation of the exposure. Sympathetic activity had increased by Day 20.

Conclusions. Interrelationships between functional parameters of the cardiovascular system and cardiac biomarkers were established, which may indicate that HDBR triggers the activation of neurohumoral and metabolic regulatory circuits in the setting of increased sympathetic autonomic modulating effects.

Keywords: head-down bed rest; heart rate variability; cardiac biomarkers; dispersion mapping; proteome; electrocardiogram; hypodynamia

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ВЗАИМОСВЯЗЬ КАРДИОМАРКЕРОВ NT-proBNP И ST2 С ФУНКЦИОНАЛЬНЫМИ ПАРАМЕТРАМИ СЕРДЕЧНО-СОСУДИСТОЙ СИСТЕМЫ В УСЛОВИЯХ АНТИОРТОСТАТИЧЕСКОЙ ГИПОКИНЕЗИИ

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Введение. Гиподинамия является одним из ключевых факторов риска сердечно-сосудистых заболеваний. Одной из моделей изучения данного состояния является антиортостатическая гипокинезия (АНОГ), которая позволяет исследовать патофизиологические изменения в сердце. Эксперименты с АНОГ выявили структурные изменения в сердце: снижение артериального давления, нарушение вегетативной регуляции и ремоделирование сосудов. Однако молекулярные механизмы этих процессов остаются малоизученными.

Цель. Изучение связи функциональных параметров сердечно-сосудистой системы с кардиомаркерами NT-proBNP и ST2.

Материалы и методы. В эксперименте участвовали 6 испытуемых-добровольцев, которые находились в условиях 21-суточной антиортостатической гипокинезии. Проведен анализ вариабельности сердечного ритма (ВСР), дисперсионного картирования (ДК ЭКГ), а также уровня NT-proBNP и концентрации белка ST2 в образцах венозной крови. Исследования выполнены за 5 дней до начала АНОГ, на 1, 10, 20 сут в условиях АНОГ и на 5 сут после экспериментального воздействия. Все вычисления были выполнены с помощью статистического программного пакета Statistica 12.

Результаты. Было показано, что концентрация белка NT-proBNP достоверно снижалась на 10 и 20 сут эксперимента, концентрация белка ST2 уменьшалась уже с первых суток гипокинезии. Показатель деполяризации левого желудочка резко возрастал в середине исследования, но возвращался к исходным значениям к 5 сут после завершения воздействия. Симпатическая активность повышалась к 20 сут.

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Выводы. Установлены взаимосвязи между функциональными параметрами сердечно-сосудистой системы и кардиомаркерами, которые могут указывать на то, что АНОГ вызывает активацию нейрогуморального и метаболического контуров регуляции на фоне повышения симпатических вегетативных модулирующих эффектов.

Ключевые слова: антиортостатическая гипокинезия; вариабельность сердечного ритма; кардиомаркеры; дисперсионное картирование; протеом; электрокардиограмма; гиподинамия

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INTRODUCTION

Hypodynamia is currently recognized to be among the main factors negatively affecting the cardiovascular system (CVS). This condition has even been declared as a twenty-first century disease due the unequivocal connection between restricted physical activity and the etiology of atherosclerosis, arterial hypertension, coronary heart disease, and some other CVS diseases. Analysis of hypodynamia effects in patients with already developed conditions is a challenging task, since the symptoms of various pathologies can disguise the effects related to hypodynamia. In this regard, the study of hypodynamia manifestations in healthy individuals appears to be an important research direction.

Head-down tilt bed rest (HDBR) is a promising model for studying the consequences of restricted physical activity in healthy individuals (Fig. 1) [1]. This model simulates not only hypodynamia but also the fluid shift toward the head, while removing the axial load from the feet [2]. Furthermore, the gravity vector changes from the anterior to the posterior part of the body, since the volunteer remains in a supine position with the bed tilted at an angle of minus 6° for several days of HDBR exposure (Fig. 1) [3].

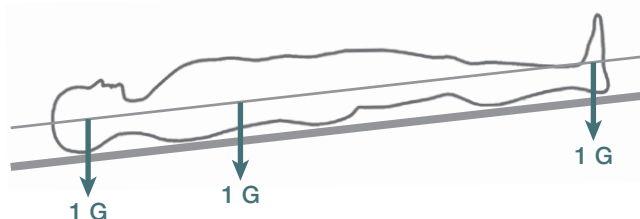


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Fig. 1. Hypodynamia model, head-down bed rest (HDBR)

HDBR studies allow changes in the CVS, primarily caused by the fluid shift in the cranial direction, to be observed [4]. Research shows that prolonged HDBR in practically healthy individuals leads to a reduction in heart chamber volumes and myocardial mass, which decreases the distensibility and functional capacity of the heart muscle [5, 6].

Chernikova et al. identified cyclic adaptive changes in CVS function during a 120-day HDBR period. Specifically, the initial stages of the experiment demonstrated a decrease in baroreflex function, a reduction in systolic arterial pressure and heart rate (HR), as well as a predominance of sympathetic influences and a weakening of the autonomous circuit of autonomic regulation¹. Under conditions of hypokinesia, chronic unloading led to remodeling of the arterial system in the legs, associated with atrophy of vascular smooth muscle. In independent studies by Palombo et al. and Platts et al., a 21-day HDBR exposure resulted in a decrease in the diastolic volume and mass of the left ventricle, as well as changes in hemodynamics and an increase in aortic stiffness [7, 8].

The HDBR influence on the morphofunctional parameters of the CVS has been comprehensively described in literature. However, the molecular mechanisms underlying these changes remain poorly understood. Such studies are particularly relevant for the development of personalized medicine approaches, since it is molecular processes that form the basis of the body's individual adaptive responses.

Cardiovascular proteomics is a promising research direction which addresses the interactions of proteins regulating the CVS function. Understanding these processes is extremely important in view of the role of autonomic and metabolic mechanisms in the body's adaptation to changing conditions, including hypodynamia. Particular attention is paid to cardiac biomarkers, such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and soluble suppression of tumorigenicity 2 (sST2),

¹ Chernikova AG. Assessment of the functional state of the body during long-term space flight based on heart rate variability analysis: abstract [dissertation]. M.: Inst. Med.-Biol. Probl.; 2010

which allow CVS dysfunction to be detected at the molecular level. These markers are capable of reflecting hidden changes preceding clinical manifestations, thus possessing high diagnostic value [9].

The sST2 soluble growth stimulation factor is expressed in cardiac tissue in response to pathological changes induced by both chronic diseases and acute injuries, manifesting the processes of ventricular remodeling and cardiac fibrosis. The NT-proBNP biomarker allows chronic obstructive pulmonary disease to be distinguished from chronic heart failure. Previous studies have established that the levels of these cardiac biomarkers change under conditions of spaceflight and parabolic flight. Changes in their levels were observed both during and after the exposure; however, within a few days, the indicators returned to their baseline values [9, 10].

Adaptive responses of the body are largely determined by nonlinear dynamic processes, including heart rate variability (HRV). Heart rate variability forms under the influence of autonomic regulation of the sinoatrial node (SA node), the bioelectrical activity of the heart generated by heart-brain interactions, and dynamic nonlinear processes of the autonomic nervous system [11]. From a physiological standpoint, HRV is an integral characteristic reflecting not only the response of cardiac structures and the CVS as a whole to external influences, but also the response of more complex neural networks at different levels of the CNS [12].

In this study, we aim to investigate a potential relationship between the functional parameters of CVS and the proBNP and ST2 cardiac biomarkers under conditions of HDBR.

MATERIALS AND METHODS

Six practically healthy male volunteers (age range 24–40 years, BMI 23.8 ± 2.7 kg/m²) were invited to

participate in a 21-day HDBR experiment [13] to simulate hypodynamia and analyze its effects.

The experiment was conducted at the Institute of Biomedical Problems of the Russian Academy of Sciences (IBMP RAS) using the Hypogravity test facility.

During the experimental exposure, electrocardiogram (ECG) recording and venous blood collection were performed on Days 1, 10, and 20 of HDBR. Baseline studies were conducted prior to and following the experiment, namely five days before the onset of HDBR and on Day 5 after the experimental exposure.

To assess bioelectrical processes in the myocardium, ECG was recorded in two standard leads over 24 h, followed by an analysis of HRV and dispersion mapping (ECG DM), which characterize the electrophysiological properties of the myocardium. The following standard HRV parameters were assessed: the standard deviation of all normal-to-normal intervals SDNN (ms) and the power spectral density in the low-frequency range LF (ms²). In addition, parameters characterizing bioelectrical stability/instability in the myocardium, including the dispersion characteristic of the PQRST complex on the ECG during the completion of left ventricular depolarization (G4) [14].

Venous blood sampling was performed in the morning in a fasting state. The level of NT-proBNP was measured using a Finecare FIA FS-113 immunoassay analyzer (Guangzhou Wondfo Biotech, China). The concentration of the ST2 protein was determined by enzyme-linked immunosorbent assay (ELISA) using a commercial Aspect Plus Kit (Critical Diagnostics, USA), with a minimum detection limit of 12.5 ng/mL [15].

Since the majority of the studied sample distributions failed to meet the normality criteria according to the Jarque–Bera test, the Spearman's rank correlation coefficient (r_s) was also applied with an assessment of the significance level (p) [16]. All calculations were

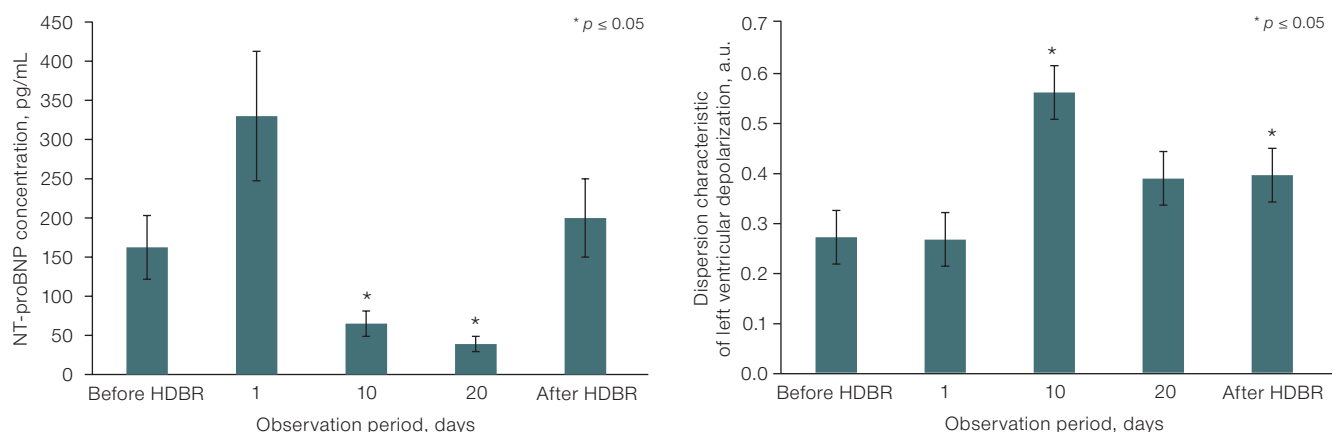


Figure prepared by the authors based on original data

Fig. 2. Dynamics of the dispersion mapping parameter and NT-proBNP protein before, during, and after head-down bed rest (HDBR)

performed using the Statistica 12 statistical software package (StatSoft, USA).

RESULTS

During the study, the dynamics of the NT-proBNP protein change were assessed in six healthy male volunteers throughout the entire research period. A significant decrease in its concentration was observed on Days 10 and 20 of the exposure compared to the baseline levels (102.6 ± 65.4 pg/mL). This may indicate a reduction in circulating blood volume by the middle of the experiment. The corresponding data are presented in Figure 2.

The values of the G4 index, reflecting the process of left ventricular depolarization, on Days 10 and 20 of HDBR increased almost threefold (to 0.6 a.u.) compared to the baseline values (0.2 a.u.). However, by Day 5 of the recovery period, the index value had corresponded to the initial (baseline) levels ($p \leq 0.05$).

The ST2 protein concentration in the volunteers ranged 21.4 ± 2.7 ng/mL five days before the onset of the experiment and was significantly decreasing ($p \leq 0.05$) starting from Day 1 of the exposure. The corresponding data are presented in Figure 3.

The SDNN index is one of the most informative HRV parameters, which reflects autonomic modulation in the SA node. It increased on Day 1 of HDBR, then gradually decreasing on Days 10 and 20 of the experiment.

The increased activity of the sympathetic division of autonomic regulation during the experiment in all subjects is illustrated in Figure 2. The low-frequency component of the HRV spectrum (LF, ms^2), which characterizes the dominance of sympathetic effects on heart rhythm, increased both on Day 20 of the HDBR exposure and during the recovery period, which may indicate the development of fatigue and stress during the final stages of the experiment.

It is worth noting that the changes in the dynamics of the ST2 protein and the LF (ms^2) index were inversely proportional.

When analyzing the results obtained using Spearman's rank correlation criterion, a relationship between CVS parameters and cardiac biomarkers was established (Table). These data show that before the HDBR exposure, the correlations between most parameters were weak and statistically insignificant. The levels of NT-proBNP and ST2 proteins showed a significant correlation with CVS parameters on Days 10 and 20 of the exposure, which likely reflects the processes of myocardial adaptation and recovery.

DISCUSSION

The BNP protein, being a heart failure marker, is capable of reducing the load on the heart. This protein dilates blood vessels, suppresses the renin and aldosterone production, improves collateral blood flow in the heart,

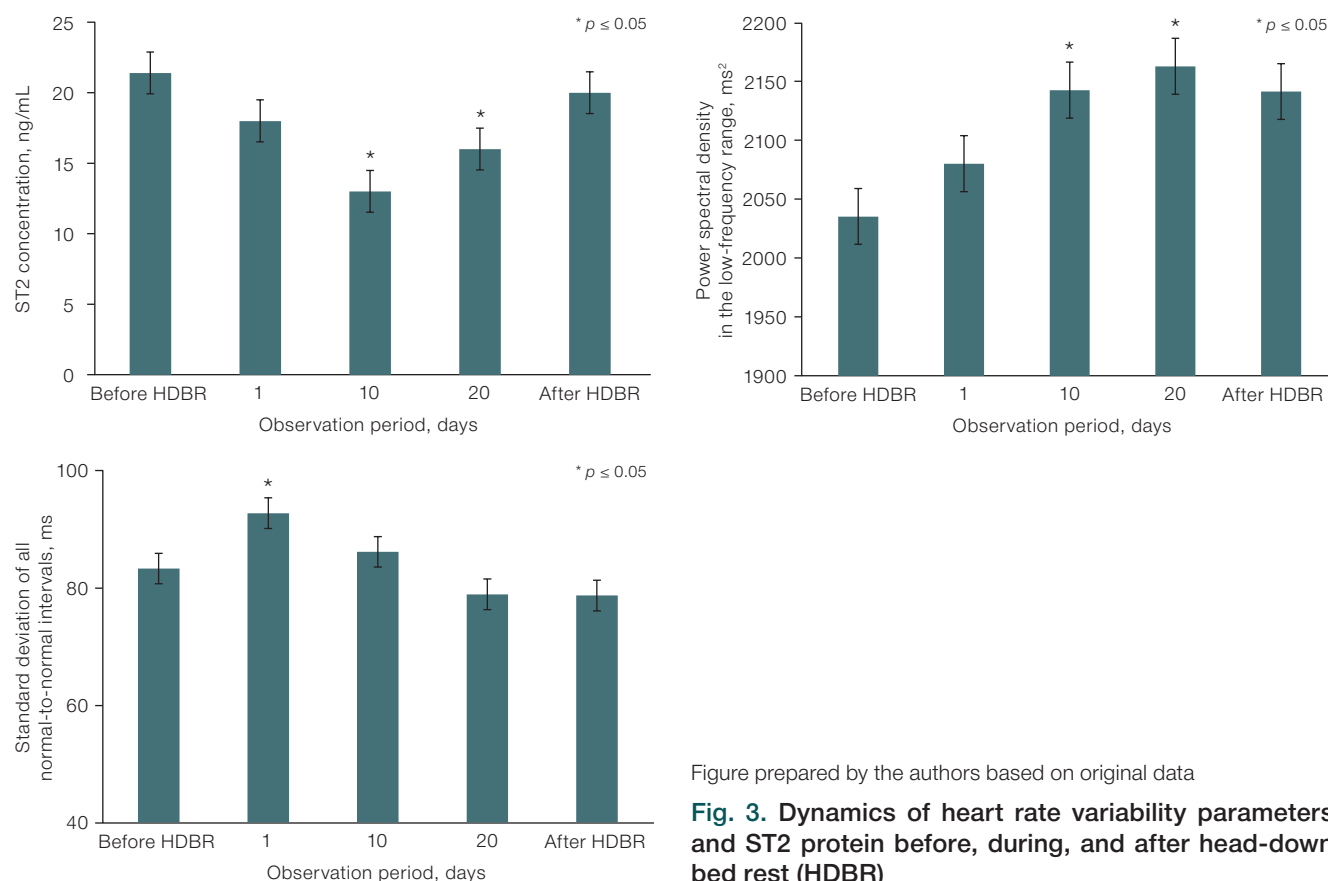


Figure prepared by the authors based on original data

Fig. 3. Dynamics of heart rate variability parameters and ST2 protein before, during, and after head-down bed rest (HDBR)

Table. Correlations between NT-proBNP and ST2 cardiac biomarkers and parameters of heart rate variability and dispersion mapping, identified in the study

Protein	Cardiovascular system (CVS) parameter	Observation duration, days				
		before HDBR	1	10	20	after HDBR
NT-proBNP	Dispersion characteristic of left ventricular depolarization, a.u.	0.26	0.45	-0.91*	-0.88*	-0.35
ST2	Standard deviation of all normal-to-normal intervals, ms	0.41	0.88*	0.64*	0.43	0.48
	Power spectral density in the low-frequency range, ms ²	-0.34	0.55	-0.88*	-0.74*	-0.26

Table compiled by the authors based on original data

Note: NT-proBNP — N-terminal pro-brain natriuretic peptide; ST2 — suppression of tumorigenicity 2; HDBR — head-down bed rest; the table contains the values of Spearman's rank correlation coefficient. (r_s); * — $p \leq 0.05$.

and reduces the load on the myocardium. As a result, a decrease in heart rate and blood pressure is observed, cardiac and vascular remodeling is reduced, and the risk of hypertrophy and thickening of the myocardial walls is lowered. It is known that the level of NT-proBNP largely depends on a set of various factors, such as circulating blood volume, changes in renal hemodynamics, and others [17].

Already on Day 1 of human exposure to HDBR conditions, a shift of body fluid components in the cranial direction was observed. On subsequent days, a gradual increase in heart rate in the setting of decreasing blood NT-proBNP concentration was noted. However, during the recovery period, the NT-proBNP level increased by almost 150 pg/mL compared to the values on Day 20 of HDBR, which likely indicates the reinclusion of gravitational load. A similar dynamic in NT-proBNP levels was recorded in other studies, particularly on Day 4 of recovery after a 7-day dry immersion [18], confirming the role of re-adaptation to orthostatic conditions in activating the natriuretic peptide response.

According to a number of studies, an increase in electrical and ion-metabolic instability is directly related to an increase in overall HRV and its parasympathetic component [19]. The electrical activity of the right atrium is largely determined by the activity of the SA node located there. Consequently, fluctuations in the ECG DM parameter may reflect processes of sinus arrhythmia in cardiac activity. Furthermore, fluctuations in venous return to the heart and changes in hemodynamic load on the atria can also lead to increased electrical instability in the atria. Regardless of the initial health status, with relatively preserved autonomous mechanisms of cardiac regulation in humans, activation of parasympathetic modulating influences on heart rhythm can lead to stabilization of the heart electrical activity, manifested as a decrease in dispersion parameters. Under

a pronounced deficit in parasympathetic modulation reserves, an impairment of atrial depolarization processes is observed, manifested by an increase in the ECG DM parameter [19].

The correlation between the NT-proBNP level and ECG DM indices identified in our study has a physiological basis. Thus, it was previously shown that during atrial fibrillation (AF), the levels of natriuretic peptide [20, 21], as well as the NT-proBNP to BNP ratio [22], increased. Rapid AF with its variable cycle length and ventricular filling time can lead to chaotic micro-regional changes in the deformation of atrial cardiomyocytes, potentially distorting and stretching local cardiomyocyte populations, causing the release of pro-BNP in the absence of increased mean intracardiac or transmural stretch pressure. Histological examination of atrial tissue in AF revealed inflammatory, hypertrophic, and fibrotic changes that could have contributed to increased expression and release of pro-BNP. These findings are consistent with the identified increased content of pro-BNP RNA messenger in atrial tissue. Together, these effects elevated the pro-BNP plasma level in AF [21].

Ozmen et al., when investigating the relationship between NT-proBNP and the frequency and severity of ventricular arrhythmia, found that NT-proBNP levels were significantly higher in the malignant arrhythmia group (Lown Class 4a and 4b arrhythmia) compared to the benign arrhythmia group (Lown Class 0–3b arrhythmia) [23].

All these studies indicate the existence of a relationship between myocardial distensibility, reflected by the NT-proBNP marker, and the rhythm of myocardial contractions (depolarization of the right atrium and left ventricle).

One possible mechanism contributing to the reduction of ventricular mass and decreased stretching of cardiomyocytes may be the interrelationship between

a potential stabilizing effect on the left ventricular end-diastolic volume and a simultaneous increase in its end-systolic volume, observed under the conditions of reduced loading during HDBR. Activation of sympathetic nerves leads to vasoconstriction of blood vessels in most organs, increased myocardial contractility, and, through its influence on the SA node, to tachycardia. It seems possible that the decrease in ST2 in the middle and at the end of the experiment activates sympathetic autonomic modulations, which, in turn, affects heart rhythm.

Furthermore, the predominance of the low-frequency component in the overall HRV spectrum may indicate an intensification of neurohumoral regulation, as well as increased activity of the vasomotor center [24]. At the same time, prolonged sympathetic stimulation of cardiac activity is accompanied by the activation of metabolic processes in the myocardium, leading to

a significant increase in oxygen and energy resource consumption [25].

CONCLUSION

In this study, we examined the dynamics of functional parameters of the CVS under conditions of HDBR, with a particular focus on molecular biochemical markers of cardiac dysfunction — proBNP and ST2. Our research has revealed an interrelationship between the functional parameters of the cardiovascular system and the proBNP and ST2 cardiac biomarkers. Under conditions of head-down bed rest, activation of neurohumoral and metabolic regulatory circuits occurs in the setting of increased sympathetic autonomic modulating effects. At the same time, the remodeling processes taking place in the heart muscle at the cardiomyocyte level are caused by an increase in their distensibility.

References

1. Puchkova AA, Shpakov AV, Baranov VM, Katuntsev VP, Stavrovskaya DM, Primachenko GK, et al. General results of the 21-day head-down bedrest study without the use of countermeasures. *Aerospace and Environmental Medicine*. 2023;57(4):31–41 (In Russ.).
<https://doi.org/10.21687/0233-528X-2023-57-4-31-41>
2. Cromwell RL, Scott JM, Downs M, Yarbough PO, Zanello SB, Ploutz-Snyder L. Overview of the NASA 70-day Bed Rest Study. *Medicine & Science in Sports & Exercise*. 2018;50(9):1909–19.
<https://doi.org/10.1249/MSS.0000000000001617>
3. Pandiarajan M, Hargens AR. Ground-Based Analogs for Human Spaceflight. *Frontiers in Physiology*. 2020;11:1–6.
<https://doi.org/10.3389/fphys.2020.00716>
4. 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/jappphysiol.00935.2015>
5. Westby CM, Martin DS, Lee SMC, Stenger MB, Platts SH. Left ventricular remodeling during and after 60 days of sedentary head-down bed rest. *Journal of Applied Physiology*. 2016;120(8):956–64.
<https://doi.org/10.1152/jappphysiol.00676.2015>
6. Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *European Journal of Applied Physiology*. 2007;101(2):143–94.
<https://doi.org/10.1007/s00421-007-0474-z>
7. Palombo C, Morizzo C, Baluci M, Lucini D, Ricci S, Biolo G, et al. Large artery remodeling and dynamics following simulated microgravity by prolonged head-down tilt bed rest in humans. *Biomed Research International*. 2015;342565.
<https://doi.org/10.1155/2015/342565>
8. Platts SH, Martin DS, Stenger MB, Perez SA, Ribiero LC, Summers R, et al. Cardiovascular adaptations to long-duration head-down bed rest. *Aviation, Space, and Environmental Medicine*. 2009;80:29–36.
<https://doi.org/10.3357/ASEM.br03.2009>
9. Jirak P, Wernly B, Lichtenauer M, Paar V, Franz M, Knost T, et al. Dynamic Changes of Heart Failure Biomarkers in Response to Parabolic Flight. *International Journal of Molecular Sciences*. 2020;21(10):3467.
<https://doi.org/10.3390/ijms21103467>
10. Pastushkova LK, Goncharova AG, Kashirina DN, Goncharov IN, Larina IM. Effect of 21-day head-down bed rest factors on the blood level of the ST2 myocardial compliance biomarker. *Human Physiology*. 2023;49:605–8.
<https://doi.org/10.1134/S0362119723600261>
11. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology*. 2014;5:1040.
<https://doi.org/10.3389/fpsyg.2014.01040>
12. Berntson GG, Bigger JTJ, Eckberg DL, Grossman P, Kaufmann PG, Malik M, et al. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology*. 1997;34(6):623–48.
<https://doi.org/10.1111/j.1469-8986.1997.tb02140.x>
13. Sundblad P, Orlov O, Angerer O, Larina I, Cromwell R. Standardization of bed rest studies in the spaceflight context. *Journal of Applied Physiology*. 2016;121(1):348–9.
<https://doi.org/10.1152/jappphysiol.00089.2016>
14. Ivanov GG, Sula AS. *Dispersion ECG mapping: theoretical foundations and clinical practice*. Moscow: Technosphere; 2009 (In Russ.).
15. Popova OV, Kashirina DN, Pastushkova LKh, Goncharova AG, Larina IM. Levels of ST2, NT-proBNP and D-dimer markers in healthy volunteer test subjects in 21-day HDBR -6° as a model of physical inactivity. *Bulletin of Experimental Biology and Medicine*. 2024;177(4):400–4.
<https://doi.org/10.1007/s10517-024-06197-7>
16. Nosovsky AM, Popova OV, Smirnov Yul. Modern technologies of statistical analysis of medical data and methods of their graphical presentation. *Aerospace and Environmental Medicine*. 2023;57(5):149–54 (In Russ.).
<https://doi.org/10.21687/0233-528X-2023-57-5-149-154>
17. Hall C. NT-ProBNP: The mechanism behind the marker. *Journal of Cardiac Failure*. 2005;11(Issue 5):S81–3.
<https://doi.org/10.1016/j.cardfail.2005.04.019>
18. Navasiolava NM, Pajot A, Gallois Y, Pastushkova LKh, Kulchitsky VA, Gauquelin-Koch G, et al. NT-ProBNP levels, water and sodium homeostasis in healthy men: effects of 7 days of dry immersion. *European Journal of Applied Physiology*. 2011;111(9):2229–37.
<https://doi.org/10.1007/s00421-011-1858-7>
19. Zenchenko TA, Poskotinova LV, Medvedeva AA, Khorseva NI. The relationship between the dynamics of heart rate variability

- ity indicators and dispersion mapping of the electrocardiogram in humans during long-term monitoring at rest. *Human Ecology*. 2012;10:16–27 (In Russ.).
<https://doi.org/10.17816/humeco17420>
20. Kristensen SL, Jhund PS, Mogensen UM, Rørth R, Abraham WT, Desai A, et al. Prognostic value of N-terminal Pro-B-type natriuretic peptide levels in heart failure patients with and without atrial fibrillation. *Circulation: Heart Failure*. 2017;10(10).
<https://doi.org/10.1161/CIRCHEARTFAILURE.117.004409>
 21. Richards M, Di Somma S, Mueller C, Nowak R, Peacock F, Ponikowski P, et al. Atrial fibrillation impairs the diagnostic performance of cardiac natriuretic peptides in dyspneic patients: Results from the BACH study (Biomarkers in ACute Heart Failure). *JACC: Heart Failure*. 2013;1(3):192–9.
<https://doi.org/10.1016/j.jchf.2013.02.004>
 22. Rørth R, Jhund PS, Yilmaz MB, Kristensen SL, Welsh P, Desai AS, et al. Comparison of BNP and NT-proBNP in patients with heart failure and reduced ejection fraction. *Circulation: Heart Failure*. 2020;13(2):e006541.
<https://doi.org/10.1161/CIRCHEARTFAILURE.119.006541>
 23. Ozmen C, Deniz A, Deveci OS, Cagiliyan CE, Celik AI, Yidiz I, et al. Association among tenascin-C and NT-proBNP levels and arrhythmia prevalence in heart failure. *Clinical and Investigation Medicine*. 2017;40(6):E219–27.
<https://doi.org/10.25011/cim.v40i6.29122>
 24. Shlyk NI. *Heart rhythm and type of regulation in children, adolescents and athletes*. Izhevsk: Publishing House “Udmurt University”; 2009 (In Russ.).
 25. Gavrilova EA. *Rhythmocardiography in sports*. St. Petersburg: North-Western State Medical University named after I.I. Mechnikov; 2014 (In Russ.).

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EFFICACY OF MEDICAL PREVENTIVE MEASURES FOR SKIN LESIONS IN MINING INDUSTRY WORKERS

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Introduction. In miners, skin lesions are associated with dysfunction of various morphofunctional skin structures: epidermis; microvasculature; nerves of the dermis and hypodermis. In order to preserve the health of the skin as an independent anatomical unit, a complex of medical preventive measures has been developed, which includes topical and systemic therapy.

Objective. To evaluate the efficacy of a complex of medical preventive measures for skin lesions in vibration-exposed miners.

Materials and methods. A prospective, single-center, open-label, randomized controlled trial was conducted before and after the implementation of medical preventive measures for skin lesions in miners. The study included vibration-exposed miners ($n = 77$) divided into a main group and a control group. Their skin condition dynamics were analyzed through clinical examination and polarized dermoscopy, as well as assessment of skin microcirculation and oxidative metabolism by means of a device combining laser Doppler flowmetry with fluorescence spectroscopy. Statistical processing was performed via generally accepted statistical methods, employing Microsoft Excel 2018 and Stat-Tech v. 4.9.5 software (StatTech, Russia).

Results. In the entire cohort of examined patients, a total of 57 (74%) individuals were diagnosed with the following skin diseases: other epidermal thickening, skin appendage diseases, allergic dermatoses, infectious skin diseases, papulosquamous dermatoses, photosensitive dermatoses, and pigmentation disorders. An analysis of clinical and dermoscopic manifestations revealed a significant positive dynamics of the pathological process in the main group ($p < 0.001$). Of all subjects, skin microcirculation dysfunction was detected in 55 (71.5%) miners. The main group exhibited a statistically significant increase in the microcirculation parameter ($p = 0.004$), a decrease in the fluorescence amplitude of reduced nicotinamide adenine dinucleotide ($p = 0.002$), and an increase in the oxidative metabolism index ($p < 0.001$).

Conclusions. The obtained results prove the efficacy of the developed complex of medical preventive measures. These measures can be implemented in addition to traditional methods of secondary prevention and rehabilitation of mining industry workers.

Keywords: miners; work-related diseases; occupational diseases; skin diseases; microcirculation; prevention

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Compliance with ethical principles: the study was approved by the Local Ethics Committee of the F.F. Erisman Federal Scientific Center of Hygiene (minutes No. 1 dated January 22, 2025) and was conducted in accordance with the generally accepted scientific principles of the Helsinki Declaration of the World Medical Association. All patients signed informed consent for treatment, as well as for the use of anonymized medical data and photographs.

Potential conflict of interest: the authors declare no conflict of interest.

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ОЦЕНКА ЭФФЕКТИВНОСТИ МЕДИКО-ПРОФИЛАКТИЧЕСКИХ МЕРОПРИЯТИЙ ПРИ ПОРАЖЕНИЯХ КОЖИ У РАБОЧИХ ГОРНОДОБЫВАЮЩЕЙ ПРОМЫШЛЕННОСТИ

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Введение. Поражения кожи горнорабочих связаны с дисфункцией различных морфофункциональных структур кожи: эпидермиса, микроциркуляторного русла и нервов дермы и гиподермы. Для сохранения здоровья кожи как самостоятельной анатомической единицы разработан комплекс медико-профилактических мероприятий, включающий наружную и системную терапию.

Цель. Оценить эффективность комплекса медико-профилактических мероприятий при поражениях кожи у горнорабочих виброопасных профессий.

Материалы и методы. Проведено проспективное одноцентровое открытое рандомизированное контролируемое исследование до и после медико-профилактических мероприятий при поражениях кожи у горнорабочих. В исследование включены горнорабочие виброопасных профессий ($n = 77$), из них выделены основная и контрольная группы. Анализ динамики состояния кожи осуществляли при проведении клинического осмотра, поляризованной дерматоскопии, оценке кожного микрокровотока и окислительного метаболизма на аппарате лазерной доплеровской флоуметрии (ЛДФ) с флуоресцентной спектроскопией. Статистическая обработка выполнена с применением общепринятых статистических методов при использовании программных пакетов Microsoft Excel 2018 и StatTech v. 4.9.5 (ООО «Статтех», Россия).

Результаты. Среди всей когорты обследованных у 57 (74%) пациентов выявлены следующие заболевания кожи: другие эпидермальные утолщения, болезни придатков кожи, аллергодерматозы, инфекционные болезни кожи, папуло-сквамозные дерматозы, фоточувствительные дерматозы, нарушения пигментации. В результате проведенных исследований в основной группе отмечали

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выраженную положительную динамику со стороны кожного патологического процесса при анализе клинико-дерматоскопических проявлений ($p < 0,001$). Среди всех испытуемых у 55 (71,5%) горнорабочих выявлена дисфункция микроциркуляции кожи. У пациентов из основной группы отмечали статистически значимое увеличение показателя микроциркуляции ($p = 0,004$), снижение амплитуды флуоресценции восстановленного никотинамидадениндинуклеотида ($p = 0,002$), рост показателя окислительного метаболизма ($p < 0,001$).

Заключение. Полученные результаты доказывают эффективность разработанного комплекса медико-профилактических мероприятий. Комплекс может быть реализован в дополнение к традиционным методам при вторичной профилактике и реабилитации рабочих горнодобывающей промышленности.

Ключевые слова: горнорабочие; профессионально обусловленные болезни; профессиональные болезни; болезни кожи; микроциркуляция; профилактика

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Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

In the mining industry, workers are at the greatest risk of developing occupational diseases as a result of exposure to industrial factors affecting various organs and tissues¹ [1].

Occupationally related dermatoses with predominant epidermal involvement are associated with exposure to chemical agents, adverse microclimatic conditions, and the physical demands of labor. These conditions manifest as skin xerosis, irritant and allergic contact dermatitis, and eczema. In miners, impaired skin barrier function plays a key role in the pathogenesis of epidermal lesions. Miners interact with chemical agents of mild potency, which, with regular exposure, irritate the skin. Such agents include weak acids and alkalis, organic solvents, heavy metal salts, and petroleum products found in cement, cutting fluids, industrial oils, etc. An additional destructive effect on the epidermis is caused by adverse microclimatic factors, such as excessively high or low temperatures and increased humidity. Regular manual lifting and movement of heavy loads also contribute to epidermal damage, as well as constant pressure on skin areas due to forced working postures² [1–5].

Exposure to occupational whole-body and local vibration, as well as adverse microclimatic conditions, causes lesions affecting the deep layers of the skin and subcutaneous tissue, which can be attributed to microcirculatory dysfunction and damage to receptors, nerve endings, and peripheral nerve

fibers. These changes represent the clinical manifestations of peripheral angiodystonic syndrome (including Raynaud's phenomenon) and vegetative-sensory polyneuropathy in vibration disease. Visually, these lesions may manifest on the skin of the distal extremities as marbling and pastiness, episodes of whitening and cyanosis, hyperhidrosis, impaired hair growth, and trophic changes³ [6].

Damage to the vessels and nerves of the dermis and hypodermis in vibration-induced conditions influences the course of occupational, work-related, and non-occupational skin diseases. The relevance and necessity of a comprehensive approach to scientifically supporting medical preventive measures for skin lesions in the vibration-exposed mining industry workers is confirmed by numerous publications on the subject. However, despite a large number of scientific studies on the impact of occupational factors in the mining industry on the human body, no studies have been conducted to examine their effect on skin lesions, with skin viewed as an anatomical unit.

One of the mechanisms for the development of skin lesions in miners is the disruption of the antioxidant system. Oxidative stress in miners as a result of their occupational activity has been described in the works of both Russian and foreign authors [7–9]. The association of oxidative stress with the onset and progression of a number of neurodegenerative diseases and inflammatory processes in various organs and tissues, as well as with microcirculatory dysfunction and premature aging, was confirmed [10, 11].

¹ On the state of sanitary and epidemiological welfare of the population in the Russian Federation in 2024: State Report. Moscow: Federal Service for the Oversight of Consumer Protection and Welfare; 2025.

² Clinical Guidelines "Contact Dermatitis", 2024. URL: https://cr.minzdrav.gov.ru/preview-cr/213_3

³ Zykov KA, Popkova AM, Smetneva NS, Igonina NP, Samoylova NV, Goloborodova IV, et al. Vibration disease: a textbook for students. Moscow: RLO MGMSU; 2022.

Using a dermoscope to examine the skin under magnification is a non-invasive way to assess the epidermis condition. Dermoscopy is widely used in the daily practice of a dermatologist/venereologist, facilitating the differential diagnosis of various skin diseases, as well as enabling the effectiveness evaluation of administered therapy [12].

Laser Doppler flowmetry (LDF) combined with fluorescence spectroscopy is an accessible, non-invasive method for diagnosing skin microcirculatory dysfunction and oxidative metabolism *in vivo*. This method relies on the Doppler shift of light scattered by blood cells [13].

In order to prevent skin lesions, initial and periodic medical examinations are conducted by a dermatologist/venereologist⁴. Mining industry enterprises also use dermatological personal protective products of cleansing, protective, and regenerative types^{5,6}. Due to the disruption of various morphofunctional skin structures in miners, it is necessary to develop a complex of medical preventive measures aimed at preserving the health of skin and subcutaneous tissue.

Moisturizers and emollients, as well as keratolytic agents, are used externally to restore the normal structure and function of the skin, epidermal barrier, and hydrolipid mantle [14, 15].

For the correction of oxidative metabolism, several studies suggest the use of systemic and topical ozone therapy. Ozone therapy stabilizes the imbalance of pro- and antioxidants, improves microcirculation, and has an immunomodulatory effect [7–11]. As a complex of medical preventive measures for skin lesions in miners, it has been proposed to administer systemic ozone therapy along with external therapy using emollients and keratolytic agents.

The study aims to evaluate the efficacy of a complex of medical preventive measures for skin lesions in vibration-exposed miners.

MATERIALS AND METHODS

A prospective, single-center, open-label, comparative study was conducted before and after the implementation of medical preventive measures for skin lesions in miners.

The study included vibration-exposed miners ($n = 77$). All subjects were males aged 34 to 60 years ($Me = 51$), with work experience of 10–37 years ($Me = 21$) at enterprises engaged in underground mining of copper-nickel ores ($n = 48$), underground ($n = 6$) and open-pit ($n = 7$) mining of ferruginous quartzites, and underground coal mining ($n = 16$). The study

participants consisted of machine operators ($n = 30$), drift miners ($n = 21$), stoper operators ($n = 11$), drillers ($n = 10$), roof bolters ($n = 2$), shotfirers ($n = 2$), and a fitter ($n = 1$).

In order to evaluate the efficacy of the complex of medical preventive measures, the subjects ($n = 77$) were divided into the following groups:

- Group 1 — main group in which the developed complex of medical preventive measures was implemented along with standard treatment ($n = 28$);
- Group 2 — control group comprising patients who received standard treatment according to clinical guidelines for the relevant nosologies ($n = 28$).

The remaining patients ($n = 21$) withdrew from the study due to contraindications to ozone therapy identified based on peripheral microcirculation indicators (hyperemic perfusion type). The developed complex of medical preventive measures included: external moisturizers (for all subjects), keratolytic agents (for patients with Grade II–III xerosis according to the dermoscopic criteria), and ozone therapy (for patients with normal and spastic perfusion).

The therapy for skin xerosis depended on its severity. Moisturizers and emollients were prescribed for external use to all subjects to be applied twice daily to the skin of the hands, shins, and feet. Additionally, moderate-to-severe xerosis patients used keratolytic agents containing 10% urea, 2% salicylic acid, and 1% lactic acid, applied twice a day externally to the skin of the hands, shins, and feet. In the absence of contraindications, intravenous administration of 500 mL ozonated saline with an ozone concentration of 1–4 mg/L was performed on alternate days in three courses using a UOTA-60-01 device (Medozon, Russia).

The mean age ($M \pm SD$) of workers in the main group was 51 years (50.68 ± 5.22 ; 95% CI 48.65–52.70), with a work experience of 22 years (22.21 ± 6.06 ; 95% CI 19.87–24.56); the mean age of workers in the control group was 53 years (52.64 ± 4.24 ; 95% CI 51.00–54.29), with a work experience of 21 years (21.14 ± 5.22 ; 95% CI 19.12–23.17). In the main and control groups, the subjects were comparable in terms of age and work experience.

All subjects had an established diagnosis of an occupational disease and underwent examination and treatment in the neurological department of the Institute of General and Occupational Pathology named after Academician of the Russian Academy of Medical Sciences A.I. Potapov (F.F. Erisman Federal Scientific Center of Hygiene, Rospotrebnadzor) in 2024–2025. According to medical records, the subjects were diagnosed with the following pathologies of occupational

⁴ Order of the Ministry of Health of the Russian Federation No. 29N dated January 28, 2021 “On Approval of the Procedure for Conducting Mandatory Preliminary and Periodic Medical Examinations of Employees, as Provided for in Part Four of Article 213 of the Labor Code of the Russian Federation, the List of Medical Contraindications to Work with Harmful and (or) Dangerous Occupational Factors, as well as to Work for which Mandatory Preliminary and Periodic Medical Examinations are Conducted.”

⁵ Order of the Ministry of Labor and Social Protection of the Russian Federation No. 767n dated October 29, 2021 “On Approval of the Unified Standard Norms for the Issuance of Personal Protective Equipment and Flushing Agents.”

⁶ GOST R 12.4.301-2018 “Occupational safety standards system. Dermatological personal protective products. General specifications.”

etiology: dorsopathy in 72 (93.5%) patients, limb polyneuropathy in 52 (67.5%), vibration disease in 42 (54.5%), lung diseases in 1 (1.3%), and sensorineural hearing loss in 1 (1.3%).

The study excluded subjects with the following decompensated diseases: diabetes mellitus, Grade 3 obesity, Grade 3 hypertension, ischemic heart disease, cardiac arrhythmias, history of acute cerebrovascular accident, blood coagulation disorders, systemic autoimmune processes, chronic kidney disease, malignant neoplasms, and other somatic diseases. Additional exclusion criteria included legume intolerance (glucose-6-phosphate dehydrogenase deficiency), allergy to ozone or components of the topical preparations used in the study, and exposure to medications capable of distorting the results.

The therapy lasted ten days, before and after which the patient underwent a clinical examination, polarized dermoscopy with a Heine Delta 30 dermoscope (Heine Optotechnik GmbH & Co. KG, Germany), and LDF with fluorescence spectroscopy with the use of a Lazma PF device (LAZMA, Russia).

When assessing the clinical picture of diagnosed skin diseases using the developed methodology, we considered the degree of erythema, the intensity of scaling, and the presence of fissures accompanied by complaints of itching, discomfort, or pain. Points were assigned to the assessed signs depending on the intensity of manifestation: from 0 (absence) to 3 (pronounced manifestations). Subsequently, the total number of points was calculated, and the degree of skin involvement was determined using a 15-point scale:

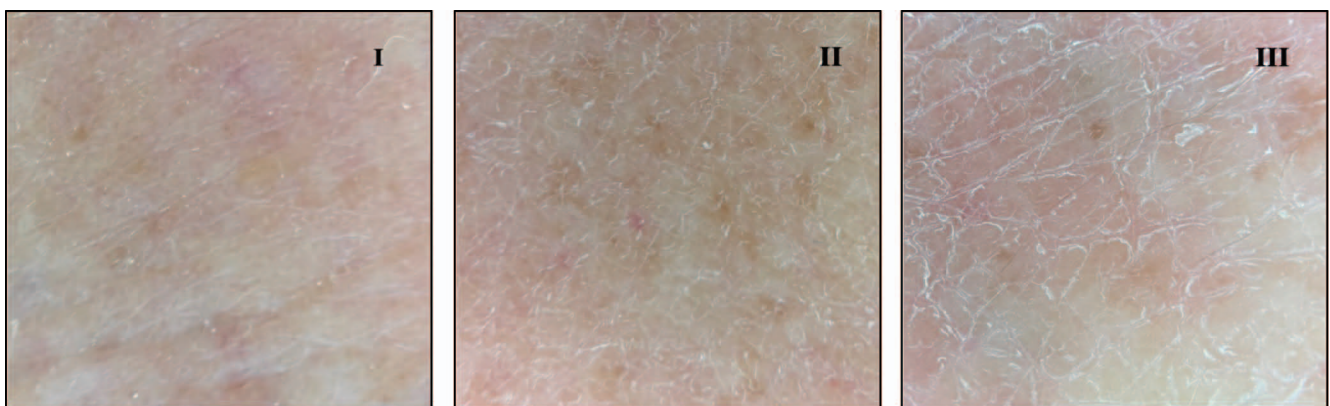
- 0–3 points: minimal changes; good condition of the skin;
- 4–6 points: moderate changes;
- 7–9 points: significant skin changes;
- 10–15 points: severe skin lesions.

The dynamics of lesion severity in skin appendage diseases and pigmentation disorders were not considered due to the impossibility of their identification according to the selected clinical diagnostic criteria (erythema, scaling, fissures, pain, discomfort, and itching).

Other epidermal thickenings, including skin xerosis of the hands, were most common among the subjects and could be occupationally related. Therefore, the efficacy of medical preventive measures was assessed by analyzing the dermoscopic signs indicating the degree of hand xerosis.

Polarized dermoscopy was performed on all subjects to assess the dynamics of xerosis severity before and after the implementation of medical preventive measures. The assessment was conducted on the dorsal hand surface at five randomly selected points, with a subsequent determination of the intermediate result. Dermoscopic criteria for assessing the severity of xerosis (Grades I–III) included the presence of scales (intensity of scaling: slight, moderate, and severe), confinement of scaling to skin furrows (within the furrows; extending beyond them), and the presence of erythema (Fig. 1) [16].

Laser Doppler flowmetry was performed on all subjects in the morning (30 min after waking; on an empty stomach and in a resting state) at an ambient temperature of 21–24 °C. In order to avoid distortions of the recorded signals, patients were asked to refrain from smoking and drinking coffee/tea for 3 h before the study. Laser Doppler flowmetry was carried out with normal blood pressure levels (from 101/61 to 139/89 mmHg), measured on the left arm with a mechanical tonometer (Korotkoff method) 15 min before the study. The patients had not taken antihypertensive or other medications for 12 h prior to the study. During the procedure, the subjects were seated, with the right forearm resting on a table at heart level. The



Dermoscopic image by the authors

Fig. 1. Dermoscopic signs indicating the degree of skin xerosis: Grade I — slight scaling confined to the skin furrows; Grade II — moderate scaling extending beyond the skin furrows; Grade III — pronounced lamellar scaling extending beyond the skin furrows, with erythema ranging from light pink to pink

analyzer was freely attached with a strap to the palmar distal phalanx of the third right-hand finger. The LDF signal was recorded for 10 min, during which time the patients were asked not to speak or make active movements.

The primary result of flowmetry is the microcirculation index, expressed in relative perfusion units (PU). Mathematically, it can be expressed as follows:

$$MI = Pc \times N_{rbc} \times V_m, \quad (1)$$

MI — microcirculation index; Pc — proportionality coefficient; N_{rbc} — number of red blood cells; V_m — mean erythrocyte velocity in the interrogated tissue volume.

Based on the microcirculation index (MI), the perfusion type was determined: normal, spastic, or hyperemic⁷ [13]. Spectroscopy was implemented using low-intensity laser radiation, enabling the fluorescence assessment of a coenzyme, reduced nicotinamide adenine dinucleotide (NADH), followed by the calculation of the oxidative metabolism index (OMI). An increase in NADH fluorescence amplitude (A_{NADH}) in tissue occurs with a decrease in antioxidant defense and mitochondrial respiration. The OMI is inversely proportional to A_{NADH} and directly proportional to nutritive microcirculation (M_{nutr}). In turn, M_{nutr} depends on the arithmetic mean value of the microcirculation index (M_{MI}) and is determined by passive (Ac, Ad) and active (Am, An) microcirculation factors [17]:

$$M_{nutr} = \frac{M_{MI} \times Am}{An + Ac}.$$

Passive regulatory factors implementing longitudinal blood flow oscillations originate outside the microcirculation system: the pulse wave from the arteries (cardiac spectrum, Ac) and the suction effect of the “respiratory pump” from the veins (respiratory spectrum, Ad). Active mechanisms of microcirculation regulation underlie transverse oscillations and are determined by endothelial (Ae), myogenic (Am), and neurogenic (An) mechanisms of vascular tone regulation⁸.

Statistical processing was performed via generally accepted statistical methods employing Microsoft Excel 2018 and StatTech v. 4.9.5 (StatTech, Russia) software. Categorical data were described in absolute numbers and percentages. Confidence intervals for percentages were calculated using the Clopper-Pearson method. Quantitative variables that follow a normal distribution were described using arithmetic means (M) and standard deviations (SD). The boundaries of the 95% confidence interval (95% CI) were indicated as a measure of representativeness for the mean values. For the correct comparison of the same indicators from groups with normal and non-normal data

distribution, quantitative parameters were described with the median (Me) and the lower and upper quartiles [$Q1$; $Q3$] using the Wilcoxon test. In the comparison of normally distributed quantitative indicators calculated for two related samples, the paired Student's t -test was used. Binary indicators characterizing two related populations were compared using McNemar's test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

All subjects ($n = 77$) underwent clinical examination, dermoscopy, and LDF with fluorescence spectroscopy. The performed clinical examination found skin diseases in 57 (74%) of all examined miners: other epidermal thickening in 33 (42.9%) workers, skin appendage diseases in 29 (37.7%), allergic dermatoses in 9 (11.7%), infectious skin diseases in 5 (6.5%), papulosquamous dermatoses in 5 (6.5%), photosensitive dermatoses in 2 (2.6%), and pigmentation disorders in 1 (1.3%).

In the main group of patients (in which the developed medical preventive measures were implemented along with standard treatment), positive dynamics were observed: a decrease in the intensity of erythema, the degree of scaling, partial epithelialization of fissures and excoriations; patients noted a reduction or regression of pain, itching, and discomfort. The examination of control group patients, who received standard treatment, also showed positive dynamics; however, in some cases, these were less significant, or a slight worsening of the skin pathology was noted. Considering the minimal skin changes, this may be due to the absence of emollients in the prescriptions of attending physicians. Noteworthy is that severe skin lesions were not diagnosed in the miners participating in the study.

The severity of skin lesions in diagnosed diseases was assessed on a scale from 0 to 15 for the convenience of analyzing dynamics. In the main group, skin diseases (excluding skin appendage diseases and pigmentation disorders) were identified in 16 subjects, with a skin lesion severity score of 5.94 ± 1.73 (95% CI [5.02; 6.86]) before therapy and 2.62 ± 1.36 (95% CI [1.90; 3.35]) after the implementation of therapeutic measures ($p < 0.001$). In the control group, skin diseases were diagnosed in 15 individuals, with a severity score of 4.60 ± 1.64 (95% CI [3.69; 5.51]) before treatment and 3.20 ± 1.86 (95% CI [2.17; 4.23]) after therapy ($p = 0.004$) (Fig. 2).

Polarized dermoscopy was performed on patients from the main and control groups ($n = 56$) (Table 1). The dynamics of dermoscopic manifestations in xerosis, which was analyzed using the Wilcoxon test, revealed a statistically significant reduction in its degree in the main group ($p < 0.001$) (Table 1).

⁷ Krupatkin AI, Sidorov VV. Functional diagnostics of the state of microcirculatory-tissue systems: Oscillations, information, nonlinearity. A guide for physicians. Stereotyped edition. Moscow: LENAND; 2022.

⁸ Ibid.

Laser Doppler flowmetry found spastic perfusion in 34 (44.2%) of all examined subjects ($n = 77$); normal, in 22 (28.6%) individuals; hyperemic, in 21 (27.3%) individuals. Due to the vasodilating effect of ozone therapy, patients with hyperemic microcirculation were excluded from the study. In order to analyze the efficacy of the complex of medical preventive measures, the microcirculation index (MI), the fluorescence amplitude of reduced nicotinamide adenine dinucleotide (A_{NADH}), and the oxidative metabolism index (OMI) were estimated.

In the main group, statistically significant changes were noted over time in MI ($p = 0.004$), A_{NADH} ($p < 0.002$) (Wilcoxon test), and OMI ($p < 0.001$) (paired Student's t -test). The main group (in which the complex of medical preventive measures was implemented) exhibited an increase in the median values of MI (15.23 and 18.33), a decrease in A_{NADH} (0.86 and 0.67), and an increase in the mean values of OMI (5.16 ± 2.19 and 6.36 ± 1.98). In the control group, the analysis of MI ($p = 0.614$), A_{NADH} ($p = 0.210$), and OMI ($p = 0.081$) over time revealed no statistically significant changes (Table 2).

According to the performed LDF, the MI dynamics noted in the main group after ozone therapy administration are associated with improved microcirculation resulting from the vasodilating effect of ozone, which is also confirmed by other studies in animals and for other pathologies [18–20]. The statistically significant OMI increase in the main group after systemic ozone therapy as compared to the control group, as well as a decrease in A_{NADH} (resulting from enhanced NADH oxidation during oxidative phosphorylation), can be attributed to the restored antioxidant system and mitochondrial respiration, as revealed by fluorescence spectroscopy [17].

This study may be limited by the uncontrolled influence of the patient's psycho-emotional state on the results obtained via the LDF method. The variability of indicators associated with the psycho-emotional state of the subjects, the patient's body position, ambient temperature, and equipment characteristics was

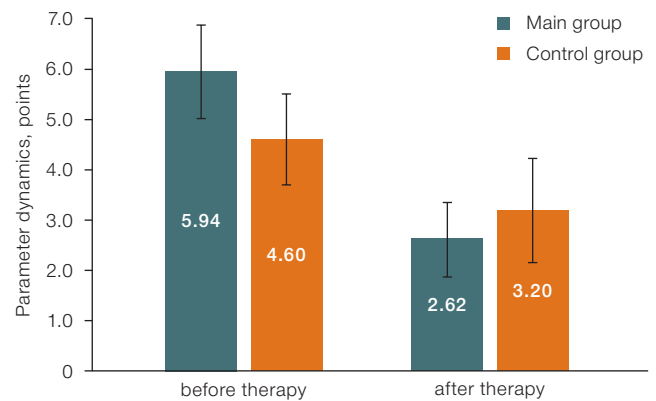


Figure prepared by the authors based on their own data

Fig. 2. Intensity of diagnosed skin lesions (on a 15-point scale) before and after the implementation of medical preventive measures in the main and control groups

reduced by establishing uniform study conditions and sample size.

The efficacy of topical therapy with emollients and keratolytic agents for skin xerosis was confirmed by foreign and Russian authors, with moisturizers also noted to be effective in other skin diseases [1, 14–16]. Numerous domestic publications examine the efficacy of topical use of ozone-oxygen gas mixture, ozonated distilled water, and various methods of systemic ozone application in infectious and autoimmune skin lesions, as well as allergic and other dermatoses [21–23]. The efficacy of ozone therapy in the treatment and prevention of vibration disease in miners was verified by laboratory and functional studies; however, these works do not assess skin condition [24, 25]. Foreign authors note the efficacy of ozone therapy in treating skin and musculoskeletal diseases while highlighting the insufficient number of studies on this topic. The available publications contain scattered information on the methods of ozone application, dosages, long-term efficacy, and safety [26, 27].

Table 1. Results of polarized dermoscopy of the skin before and after the implementation of medical preventive measures

Grade of xerosis	Main group*		Control group*	
	Before therapy	After therapy	Before therapy	After therapy
0	42.9 (24.5–62.8)	71.4 (51.3–86.8)	53.6 (33.9–72.5)	53.6 (33.9–72.5)
I	25.0 (10.7–44.9)	21.4 (8.3–41.0)	17.9 (6.1–36.9)	28.6 (13.2–48.7)
II	28.6 (13.2–48.7)	7.1 (0.9–23.5)	25.0 (10.7–44.9)	14.3 (4.0–32.7)
III	3.6 (0.1–18.3)	0	3.6 (0.1–18.3)	3.6 (0.1–18.3)

Table compiled by the authors based on their own data

Note: Data are presented as relative values and 95% confidence intervals; * — $p < 0.001$ for the main group and $p = 0.317$ for the control group in the Wilcoxon test.

Table 2. Results of laser Doppler flowmetry combined with fluorescence spectroscopy before and after the implementation of medical preventive measures

Parameters	Main group (n = 28)			Control group (n = 28)		
	Before therapy	After therapy	Statistical significance level, p	Before therapy	After therapy	Statistical significance level, p
MI, PU ¹	15.23 [13.28; 20.94]	18.33 [14.29; 21.96]	0.004	15.88 [13.56; 20.44]	14.73 [13.49; 20.36]	0.614
A _{NADH} , a.u. ¹	0.86 [0.71; 0.99]	0.67 [0.58; 0.88]	0.002	0.93 [0.82; 1.01]	0.98 [0.89; 1.04]	0.210
OMI, a.u. ²	5.16 ± 2.19 (4.31–6.01)	6.36 ± 1.98 (5.60–7.13)	< 0.001	4.94 ± 1.20 (4.48–5.41)	4.40 ± 1.22 (3.93–4.88)	0.081

Table compiled by the authors based on their own data

Note: ¹ — data are presented as median and interquartile range (Me [Q1; Q3]), p-value in the Wilcoxon test; ² — data are presented as arithmetic means and standard deviations with a 95% confidence interval ($M \pm SD$ (95% CI)), p-value in the paired Student's t-test; MI — microcirculation index; A_{NADH} — NADH fluorescence amplitude; OMI — oxidative metabolism index; a.u. — arbitrary units; PU — perfusion units

The published data on the application of ozone in skin diseases and in vibration disease indicate the relevance of ozone therapy for skin lesions in vibration-exposed miners. The obtained data do not contradict the results of previous studies, complementing them within the field of occupational medicine.

CONCLUSION

The developed complex of medical preventive measures was found to be effective in skin and subcutaneous tissue lesions caused by occupational factors affecting vibration-exposed miners. The obtained results show their positive impact on the morphofunctional structures of skin and subcutaneous tissue. The objective and subjective manifestations of skin lesions diagnosed during the study improved on average by 55.89% in the main group and by 30.43% in the control group; the microcirculation index increased by 20.35% in the main group, while in the control group it

decreased slightly (by 7.24%). These figures indicate a more significant reduction in the clinical manifestations of skin lesions in the main group compared to the control group, as well as a microcirculation improvement in the main group.

In the early stages of development, epidermal lesions are easily diagnosable and potentially reversible, unlike vascular-neurogenic dermal lesions. The obtained data confirm the need for timely diagnosis of skin xerosis and the use of topical agents for the treatment and prevention of epidermal lesions in miners in order to prevent more severe skin diseases and improve the quality of life. Also, reducing dryness is important in the treatment of most skin diseases, which also ensures the efficacy of measures concerning other skin conditions.

The developed complex of medical preventive measures can be implemented in addition to traditional methods in the treatment and prevention of skin lesions in mining industry workers.

References

1. Yatsyna IV, Astakhova IV. Analysis of skin lesion formation and organisation of medical and preventive measures in workers og mining industry. *Occupational Medicine and Human Ecology*. 2025;323–38 (In Russ.).
<https://doi.org/10.24412/2411-3794-10.24412/2411-3794-2025-10302>

2. Dear K, Toholka R, Nixon R. Occupational skin disease in mining: an Australian case series. *Arch Environ Occup Health*. 2021;76(8):504–10.
<https://doi.org/10.1080/19338244.2020.1857674>

3. Mu L, Sun A, Chen Y, Chen H, Li J, Linghu B, et al. Vascular response to the microcirculation in the fingertip by local vibration with varied amplitude. *Front Bioeng Biotechnol*. 2023;11:1197772.
<https://doi.org/10.3389/fbioe.2023.1197772>

4. Ture HY, Lee NY, Kim NR, Nam EJ. Raynaud’s Phenomenon: A Current Update on Pathogenesis, Diagnostic Workup, and Treatment. *Vasc Specialist Int*. 2024;40:26.
<https://doi.org/10.5758/vsi.240047>

5. Ufimtseva MA, Nikolaeva K.I, Beresneva TA, Mylnikova ES, Shubina AS, Sorokina KN, et al. Modern approach to the diagnosis of occupational skin diseases in workers of industrial enterprises of the Sverdlovsk region. *Modern problems of science and education*. 2021;2 (In Russ.).
<https://doi.org/10.17513/spno.30580>

6. Berg AV, Penina GO. Clinical and functional characteristics of disabled people due to diseases of the peripheral nervous system at working age. *Klinicheskaya meditsina*. 2021;99(2):108–14 (In Russ.).
<https://doi.org/10.30629/0023-2149-2021-99-2-108-114>
7. Hu HY, Zhang B, Yang LY, Zhou L, Wang Y, Wang F. PAHs exposure and its association with oxidative stress and neurotransmitter levels in coal miners. *Chinese Journal of Occupational Health and Occupational Diseases*. 2022;40(10):727–32.
<https://doi.org/10.3760/cma.j.cn121094-20210315-00150>
8. Ullah I, Zahid M, Jawad M, Arsh A. Assessment of DNA damage and oxidative stress among traffic conductors and coal miners. *Pak J Med Sci*. 2021;37(2):499–502
<https://doi.org/10.12669/pjms.37.2.2848>
9. Zheglova AV. Improving the Methodology for assessing occupational risk in Workers under the Influence of Physical Factors. *Gigiena i Sanitariya (Hygiene and Sanitation, Russian journal)*. 2021;100(9):975–79 (In Russ.).
<https://doi.org/10.47470/0016-9900-2021-100-9-975-979>
10. Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladăncu O, Roza E, et al.. An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. *Int J Mol Sci*. 2022;23(11):5938.
<https://doi.org/10.3390/ijms23115938>
11. Mezentsev YA, Osipova OA. Review of current information impact of oxidative stress on premature aging. *Current problems of health care and medical statistics*. 2022;5:249–69 (In Russ.).
<https://doi.org/10.24412/2312-2935-2022-5-249-269>
12. Szebényi J, Légrádi M, Németh C, Joura MJ, Gyulai R, Lengyel Z. Dermatoskopie entzündlicher Hauterkrankungen [Dermoscopy of inflammatory skin diseases]. *Die Dermatologie*. 2023;74(4):232–42.
<https://doi.org/10.1007/s00105-023-05122-9>
13. Kralj L, Lenasi H. Wavelet analysis of laser Doppler microcirculatory signals: Current applications and limitations. *Front Physiol*. 2023;13:1076445.
<https://doi.org/10.3389/fphys.2022.1076445>
14. Proksch E, Berardesca E, Misery L, Engblom J, Bouwstra J. Dry skin management: practical approach in light of latest research on skin structure and function. *J Dermatolog Treat*. 2020;31(7):716–22.
<https://doi.org/10.1080/09546634.2019.1607024>
15. Fluhr JW, Muguet V, Christen-Zaech S. Restoring Skin Hydration and Barrier Function: Mechanistic Insights Into Basic Emollients for Xerosis Cutis. *Int J Dermatol*. 2025;64(1):5–12.
<https://doi.org/10.1111/ijd.17790>
16. Hu SC, Lin CL, Yu HS. Dermoscopic assessment of xerosis severity, pigmentation pattern and vascular morphology in subjects with physiological aging and photoaging. *Eur J Dermatol*. 2019;29(3):274–80.
<https://doi.org/10.1684/ejd.2019.3555>
17. Luo X, Li R, Yan LJ. Roles of Pyruvate, NADH, and Mitochondrial Complex I in Redox Balance and Imbalance in β Cell Function and Dysfunction. *J Diabetes Res*. 2015;2015:512618.
<https://doi.org/10.1155/2015/512618>
18. VOLGAMEDSCIENCE. Conference proceedings of the VI All-Russian conference of young scientists and students with international participation. Nizhnii Novgorod; 2020 (In Russ.).
19. Peretyagin SP, Struchkov AA, Kostina OV, Martusevich AK, Pogodin IE, Solov'eva AG i dr. Ozonoterapiya kak dopolnitel'nyi metod v kompleksnom lechenii ozhogovoi bolezni. Ozone therapy as an additional method in the comprehensive treatment of burn injury. *Bioradicals and Antioxidants*. 2022;9(1–2):18–34 (In Russ.).
EDN: [KWMJGA](https://www.edn.ru/KWMJGA)
20. Kulikov AG. Ozonotherapy: microhemodynamical aspects. *Russian Journal of Physiotherapy, Balneology and Rehabilitation*. 2012;11(3):3–8 (In Russ.).
EDN: [PGPCGF](https://www.edn.ru/PGPCGF)
21. Kosheleva IV, Bitkina OA, Pershina KS, Kontorshchikova KN, Bitkina EV. Review of modern world experience of using ozone therapy in dermatology. *Farmateka*. 2024;31(5):46–52 (In Russ.).
<https://doi.org/10.18565/pharmateka.2024.5.46-52>
22. Krasavina EK, Yatsyna IV. Correction of cytochemical changes in patients with occupational allergodermatosis. *Russian Journal of Occupational Health and Industrial Ecology*. 2019;59(9):661–2 (In Russ.).
<https://doi.org/10.31089/1026-9428-2019-59-9-661-662>
23. Olisova OY, Severgina LO, Martynenko DM, Kogan EA, Demura TA. The role of ozone therapy in the treatment of dermatoses associated with COVID-19. *Effective Pharmacotherapy*. 2025;21(30):20–8 (In Russ.).
<https://doi.org/10.33978/2307-3586-2025-21-30-20-28>
24. Miryutova NF, Zaitsev AA, Panacheva LA, Zaikina EA. Efficiency of therapeutically applied physical factors for vibration disease caused by exposure to local vibration (review of literature). *Russian Journal of Occupational Health and Industrial Ecology*. 2017;3:59–64 (In Russ.).
EDN: [YGBNXH](https://www.edn.ru/YGBNXH)
25. Hygienic science and practice in solving the issues of sanitary and epidemiological well-being of the population in the central regions of Russia. Conference proceedings. Lipetsk; 2003 (In Russ.).
26. Liu L, Zeng L, Gao L, Zeng J, Lu J. Ozone therapy for skin diseases: Cellular and molecular mechanisms. *Int Wound J*. 2023;20(6):2376–85.
<https://doi.org/10.1111/iwj.14060>
27. Modena DAO, Ferreira RC, Froes PM, Rocha KC. Ozone Therapy for Dermatological Conditions: A Systematic Review. *J Clin Aesthet Dermatol*. 2022;15(5):65–73.
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