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**Коллектив ФНКЦ РР
и редакция журнала «Общая реаниматология»
поздравляют директора ФНКЦ РР,
Академика РАН Гречко А. В.
и первого заместителя директора ФНКЦ РР,
д. м. н. Яковлева А. А.
с присуждением Государственной премии
Российской Федерации имени Маршала Советского Союза
Г. К. Жукова в области военной науки**

Указом Президента Российской Федерации Владимира Владимировича Путина от 27 апреля 2026 г. Директор ФНКЦ РР, Академик РАН Гречко А. В. и первый заместитель директора ФНКЦ РР, д. м. н. Яковлев А. А. удостоены Государственной премии Российской Федерации имени Маршала Советского Союза Г. К. Жукова в области военной науки за создание технологий и алгоритмов реабилитации, абилитации и ресоциализации для участников Специальной военной операции.

Премия имени легендарного полководца, героя Советского Союза Георгия Константиновича Жукова, учрежденная в ознаменование Победы в Великой Отечественной войне (Указ Президента Российской Федерации от 28 октября 1996 г. № 1499), является одной из высших государственных наград в области оборонной науки и техники. Государственная премия Российской Федерации имени Маршала Советского Союза Г. К. Жукова в области военной науки присуждается Президентом Российской Федерации одному наиболее выдающемуся научному коллективу один раз в год. Быть удостоенным этой награды — значит не просто внести вклад в науку, а совершить подвиг во имя Родины и укрепления ее Вооруженных Сил. Государственная премия Российской Федерации им. Маршала Советского Союза Г. К. Жукова в области военной науки присуждается за значимые научные работы, исследования и открытия, существенно обогатившие отечественную военную науку и внесшие значительный вклад в укрепление обороноспособности страны и сохранение исторической памяти о событиях отечественной военной истории, за создание фундаментальных военно-теоретических трудов по вопросам обеспечения обороны страны и безопасности госу-



дарства, а также строительства Вооруженных Сил Российской Федерации и других войск.

Государственная премия Российской Федерации имени Маршала Советского Союза Г. К. Жукова в области военной науки, врученная руководителям ФНКЦ РР — высшее признание их заслуг, имеющих стратегическое значение для обороноспособности страны и защиты ее граждан!

*Поздравляем директора ФНКЦ РР,
Академика РАН А. В. Гречко
и первого заместителя директора —
руководителя НИИ реабилитологии
им. проф. Пряникова И. В. ФНКЦ РР,
д. м. н. А. А. Яковлева
с высокой наградой!*

К юбилею профессора Валерия Владимировича Лихванцева

Валерий Владимирович Лихванцев родился 7 апреля 1956 г. В 1979 г. с отличием окончил лечебный факультет Рязанского медицинского института имени академика И. П. Павлова. Профессиональный путь начал врачом в лечебных учреждениях РСФСР. В 1982 г. прошел первичную специализацию по анестезиологии-реаниматологии и работал врачом в различных учреждениях страны. В 1986 г. перешел в Институт хирургии им. А. В. Вишневского, где последовательно прошел все ступени — от врача до главного научного сотрудника.

В 1991 г. защитил докторскую диссертацию, а в 1996 г. получил звание профессора по специальности «анестезиология-реаниматология». В 1999–2003 гг. был председателем Московского научного общества анестезиологов-реаниматологов. С 2004 по 2020 гг. занимал руководящие должности в Мединцентре Главного управления по обслуживанию дипломатического корпуса при Министерстве иностранных дел Российской Федерации (ГлавУпДК при МИД РФ), Научно-исследовательском институте общей реаниматологии им. В. А. Неговского, Московском областном научно-исследовательском клиническом институте им. М. Ф. Владимирского. В настоящее время работает в Федеральном научно-клиническом центре реаниматологии и реабилитологии.

Профессор В. В. Лихванцев — один из ведущих анестезиологов-реаниматологов страны, внесший существенный вклад в развитие специальности. Он автор 335 научных трудов и более 10 учебных пособий. Под его руководством активно развивается научная школа доказательной анестезиологии и реаниматологии, подготовлены 38 кандидатов и 8 докторов медицинских наук, создана первая в РФ открытая база данных реанимационных пациентов (RICD) — мощный национальный исследовательский ресурс. Им впервые в России опубликованы методические рекомендации



по принципам анализа данных на промежуточных этапах исследований.

Валерий Владимирович успешно сочетает научную и педагогическую деятельность, читает лекции и ведет семинары в Сеченовском университете. Входит в редколлегии журналов «Анестезиология и реаниматология», «Вестник анестезиологии и реаниматологии», является редактором международного журнала «Frontiers in Anesthesiology». Действительный член и участник правления ведущих российских профессиональных ассоциаций (МНОАР), а также действительный член Европейского общества анестезиологии и интенсивной терапии (ESAIC) и Европейского общества кардиоторакальной анестезиологии и интенсивной терапии (EACTAIC).

Коллеги и ученики Валерия Владимировича особенно ценят редкое сочетание высокой требовательности и справедливости. Он никогда не оставляет без внимания чужую мысль, всегда открыт для диалога, новых идей и конструктивной критики. При всей своей занятости он щедро делится опытом, передает знания молодому поколению, поддерживает в начинаниях. Для многих он не просто наставник, а пример врача и ученого, которому хочется соответствовать.

Редакция журнала «Общая реаниматология», друзья, коллеги и ученики сердечно поздравляют Валерия Владимировича Лихванцева с юбилеем, желают крепкого здоровья и новых достижений в медицинской науке.

Instructions for Authors Before Manuscript Submission (v. April 21, 2025)

Before submitting your manuscript for review, make sure that the file contains all required information in either Russian or English, that all sources are properly cited, that the complete set of figures and tables is included, and that all quotations are properly formatted.

1. Checklists for Manuscript Preparation

The editors of General Reanimatology recommend to authors to use the following checklists when preparing articles and other submissions. These tools are developed and regularly updated by leading international health research organizations such as EQUATOR, Enhancing the Quality and Transparency of Health Research; SWIHM, Scientific Writing in Health & Medicine <https://www.swihm.com/course>:

- For manuscripts reporting **randomized clinical trials**: use the CONSORT 2010 checklist of information to include when reporting a randomized trial
- For manuscripts reporting **non-experimental (observational) studies**: use The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies
- When preparing a **systematic review and meta-analysis**: use PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)

We recommend structuring the abstract of the **thematic review** as follows:

- Scope of the problem (1–3 sentences from the introduction).
- Aim of the review (identical wording in the abstract and introduction).
- Number of studies included, selection criteria and databases used for source selection.
- Specific questions addressed in the main body of the review, corresponding to the identified subheadings.
- Limitations of the studies in relation to the questions addressed.
- Conclusion (a brief version of the conclusion from the main body of the review).

- When writing a **clinical case report**: use The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development, <https://www.carestatement.org/checklist>; scientific description of a clinical case following the recommendations of Scientific Writing in Health & Medicine, <https://www.swihm.com/course>

- When preparing a **manuscript reflecting the results of qualitative research**: use SRQR (Standards for reporting qualitative research)

- When preparing a **manuscript reflecting the results of prognostic studies**: use STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies

2. Research Ethics and Patient Consent

Biomedical research involving human subjects must adhere to the principles outlined in the Declaration of Helsinki (World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. DOI: 10.1001/jama.2013.281053). Such studies must be reviewed and approved in advance by the Ethics Committee(s) affiliated with the institution(s) where the research is being conducted.

All patients and volunteers have a right to privacy, which must not be violated without obtaining informed consent (IC) for participation in the study and for the anonymized publication of its results. The IC must be signed by the participants themselves or by their legal representatives. In cases where the patient is identifiable, the manuscript must be shown to the patient or the patient's legal representative prior to publication and IC for publication must be obtained.

Information regarding ethical approval and availability of IC must be included in the Materials and Methods section. This should include the Protocol number with the Ethics Committee decision, the date of approval, and a statement confirming that IC was obtained.

3. Animal studies

Animal studies must be reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments, <https://arriveguidelines.org/>).

4. Communication Between Authors and the Editorial Office

All correspondence from Authors to the Editorial Office must be sent in writing via official e-mail or the website: **journal_or@mail.ru** or **www.reanimatology.com**.

Manuscript submission is done in the Author's personal account after registration and login at the journal's website: **<http://www.reanimatology.com/>**.

For inquiries regarding manuscript preparation, submission, peer review, or the current and final status of a submission, Authors may contact the Managing editor.

In the event of a dispute or withdrawal of a manuscript, Authors must submit a formal written statement to the Editor-in-Chief, clearly and concisely stating the nature of the issue and supporting arguments.

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ОБЩАЯ РЕАНИМАТОЛОГИЯ OBŠAÂ REANIMATOLOGIÂ

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Predictive Performance of Rapid Scoring Scales in Intensive Care Patients with COVID-19

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Summary

COVID-19 remains one of the most significant causes of hospital admissions and deaths, frequently associated with septic complications. Reliable and simple prognostic tools are required to identify patients at high risk of developing sepsis and death early on.

Aim of the study. To evaluate the prognostic ability of the REMS, NEWS, NEWS2, qSOFA, and SIRS criteria in predicting sepsis, septic shock, and mortality in patients with COVID-19.

Materials and methods. A retrospective study was performed on 870 patients with confirmed COVID-19 who were hospitalized in anesthesiology and intensive care units. REMS, NEWS, NEWS2, qSOFA, and SIRS scores were calculated for each patient upon admission. The predictive ability of the scales was evaluated using ROC analysis, temporal associations were assessed using the Cox proportional hazards model, and the cumulative risk of outcome was assessed using the Kaplan–Meier method.

Results. The REMS scale demonstrated satisfactory predictive ability for mortality and septic shock (AUC=0.780 and 0.724, respectively) and unsatisfactory for sepsis (AUC=0.677), $p < 0.0001$. The NEWS, NEWS2, qSOFA, and SIRS scales showed unsatisfactory results for all outcomes. The Kaplan–Meier analysis showed that patients with high REMS scores developed sepsis or septic shock earlier and had shorter survival ($p < 0.0001$). The Cox model identified REMS as the scale with the highest risk ratio (HR 1.215; 95% CI 1.178–1.254) and concordance index (C=0.656).

Conclusion. The REMS, NEWS, NEWS2, qSOFA, and SIRS rapid scales have a statistically significant prognostic value, but only the REMS provides a satisfactory accuracy in predicting septic shock and death in patients with COVID-19. The study was limited by its single-center and retrospective design.

Keywords: COVID-19 mortality risk; rapid scales; REMS; NEWS; NEWS2; qSOFA; SIRS criteria; sepsis; septic shock

Conflict of interest. The authors declare no conflict of interest.

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Introduction

According to the World Health Organization, about 404 000 deaths related to SARS-CoV-2 (coronavirus 2-related severe acute respiratory syndrome) were registered in the Russian Federation at the end of December 2025 [1]. Although the COVID-19 pandemic had actually ended by the end of 2025, and COVID-19 infection incidence had decreased, sepsis and septic shock complicated by multiple organ failure remain common in patients with severe COVID-19 [2]. Despite high mortality rates among patients with these complications [3–5], there are still no simple and validated tools for early prediction of sepsis and septic shock in patients with COVID-19 in the Russian Federation.

The world literature describes the widespread use of early warning scores, i. e., rapid scoring systems/scales (RSS) such as the quick Sequential Organ Failure Assessment (qSOFA) [6, 7], National Early Warning Score (NEWS) and NEWS2 [8, 9], Rapid Emergency Medicine Score (REMS) [10], and Systemic Inflammatory Response (SIRS) criteria [11] for rapid assessment of disease severity in critically ill patients. However, RSS comparative prognostic performance in patients with COVID-19, not only in relation to sepsis and/or septic shock, but also in relation to mortality, has not been sufficiently studied. This necessitates a comparative analysis of several rapid scoring systems in the same cohort of patients and comparison of their diagnostic characteristics.

In this regard, the aim was to compare the prognostic value of the REMS, NEWS, NEWS2, qSOFA scoring systems and criteria for predicting the development of sepsis, septic shock, and in-hospital mortality in emergency department patients with COVID-19, and to build a proportional hazards model to assess the potential interdependence between score numbers and time to development of an adverse outcome.

Materials and Methods

A retrospective observational study was conducted using data from electronic medical records (EMR) of patients who were hospitalized between April 2020 and January 2022 in the Department of Anesthesiology and Intensive Care /Emergency Department (ICU/ED) of the Krasnodar Clinical Hospital No. 2. The study was approved by the local ethics committee (protocol No. 104 dated 22.10.2021).

Totally 986 patients data were analyzed, 116 were excluded according to the exclusion criteria. For the final analysis, the data of 870 patients were used, of which 492 (56.6%) died, 516 (59.3%) developed sepsis, and 429 (49.3%) developed septic shock.

Verification of SARS-CoV-2 infection (ICD-10 code U07.1) was based on recorded diagnosis confirmed by positive PCR tests in patients' EMR (according to Rospotrebnadzor). The outcomes were recorded based on the date of the event in the EMR. If a lower respiratory tract infection (COVID-19-associated pneumonia) was recorded in patient's EMR, it was considered as potential cause of sepsis or septic shock. The diagnoses were used as they were reported by attending physicians, without retrospective reclassification.

The study included patients over the age of 18 who were admitted ICU/ED with confirmed or suspected diagnosis of coronavirus infection at the time of admission. Patients were excluded from the study if necessary for analysis information lacked in their medical records (physical and laboratory parameters, and outcome data); if they had hematological diseases; if they had surgery (except for tracheostomy) prior to admission; if they had malignant neoplasms (MN); if they were part of the pre-mortality cohort; if they were receiving programmable hemodialysis; if they had CHF stage 2B or higher; if they were in the acute phase of stroke or traumatic brain injury; and if they were on mechanical ventilation at the time of admission to ICU/ED.

Out of total 870 patients analyzed with median age of 66.0 (55.0–75.0) years 414 (47.6%) were men. All patients were managed according to the temporary guidelines of the Ministry of Health of the Russian Federation [12] and the methodological recommendations of the Federation of Anesthesiologists-Reanimatologists of Russia (FAR) [13].

The study database included demographic (gender and age) data, records of sepsis, septic shock and SARS-CoV-2 infection diagnoses, patients outcome data (discharge from the hospital or death), and info on other clinical parameters necessary for implementing the qSOFA, NEWS, NEWS2, REMS, and SIRS RSS. The scores were calculated retrospectively based on clinical data recorded in the first minutes of ICU/ED admission.

Nonparametric methods were used for statistical data analysis due to the non-normal distribution of most quantitative variables (according to the Shapiro–Wilk test). Quantitative variables were presented as median and interquartile range (*Me* (Q1; Q3)); qualitative variables were presented as absolute values (*n*) and percentages.

Quantitative variables were compared using the Mann–Whitney test. Categorical variables were compared using the χ^2 test; Fisher's exact test was used for small expected frequencies.

The predictive performance of the REMS, NEWS, NEWS2, qSOFA scoring systems, and SIRS was evaluated using ROC analysis to determine the area under the curve (AUC), 95% confidence interval (95% CI), sensitivity, specificity, and Youden index (J) to identify the optimal threshold value. According to the commonly accepted Hosmer–Lemeshow classification [14], the predictive power was assessed as follows:

- as unsatisfactory at AUROC < 0.7;
- satisfactory at AUROC = 0.7–0.8;
- good at AUROC = 0.8–0.9;
- excellent at AUROC > 0.9.

Sensitivity or specificity values of ≥ 0.90 were considered high, of 0.80–0.89 — satisfactory, and < 0.80 were considered moderate or low.

Cumulative risk and time to adverse outcomes (sepsis, septic shock, death) were assessed using the Kaplan–Meier method with log rank test to compare curves. To clarify the effect of score number on different RSS on the risk of adverse outcome, the Cox proportional hazards model was used with the calculation of hazards ratios (HR) and 95% CI. The proportional hazards (PH) assumption was tested using the Schoenfeld residue test. Due to detected violation of PH assumption for the REMS scoring system, the Cox model with the time-dependent REMS effect was used.

Patients were stratified according to the threshold values of the RSS, determined by the ROC analysis.

Statistical significance was accepted at $p < 0.05$. The data were processed using the MedCalc 20.027 program (MedCalc Software Ltd., Belgium). R version 4.5.1 was used to test the proportional hazards assumption using Cox regression.

The Results of the Study

The patient selection scheme is presented in Fig. 1.

Table 1 presents a comparison of baseline parameters analyzed in the rapid clinical scoring systems for surviving and non-surviving patients.

Patients who died were, on average, older than the survivors, more often had impaired consciousness, higher respiratory rate, and lower pulse oximetry values, indicating the need in oxygen support. Sepsis and septic shock were more common in non-survivors.

Results of ROC-analysis of the mortality, sepsis and septic shock prediction.

ROC-analysis results of the rapid scoring systems performance in mortality prediction are presented in Table 2.1.

The REMS scoring system demonstrated a satisfactory predictive power (AUC 0.780; 95% CI 0.751–0.807, $p < 0.0001$). The optimal threshold value (cut-off) was > 4 scores with a satisfactory sensitivity of 81.1% and a low specificity of 61.5% (Youden J index 0.425).

The predictive performance of the NEWS (AUC 0.678), NEWS2 (AUC 0.678), qSOFA (AUC 0.618) scoring systems, and SIRS (AUC 0.578) was unsatisfactory.

The results of the ROC analysis of RSS performance in prediction of sepsis are presented in Table 2.2.

All RSS showed unsatisfactory accuracy in prediction of sepsis.

The results of the ROC analysis of rapid scoring systems performance in prediction of septic shock are presented in Table 2.3.

The REMS showed the highest area under the ROC curve (AUC 0.724; 95% CI 0.693–0.754, $p < 0.001$), demonstrating the best combination of moderate sensitivity (79.75%) and low specificity (52.9%).

The NEWS (AUC=0.642), NEWS2 (AUC=0.639), qSOFA (AUC=0.592), and SIRS (AUC=0.574) RSS showed poor predictive ability.

The comparative ROC curves of the rapid scoring systems in predicting outcomes are presented in Fig. 2. REMS had statistically significant differences in AUROC compared to the other rapid scoring systems ($p < 0.001$).

The analysis of survival data: the Kaplan–Meier method.

Kaplan–Meier analysis was performed only for the REMS due to unsatisfactory discrimination capacity of other rapid scoring systems.

For Kaplan–Meier survival analysis, patients were stratified into high- and low-risk groups according to RSS thresholds for different outcomes determined by ROC analysis.

For REMS, the cutoff point was > 4 for all outcomes; 325 (37.4%) patients were in the low-risk group, and 545 (62.6%) were in the high-risk group.

The time-to-endpoint/event (TTE) analysis was conducted within the entire hospital stay. The

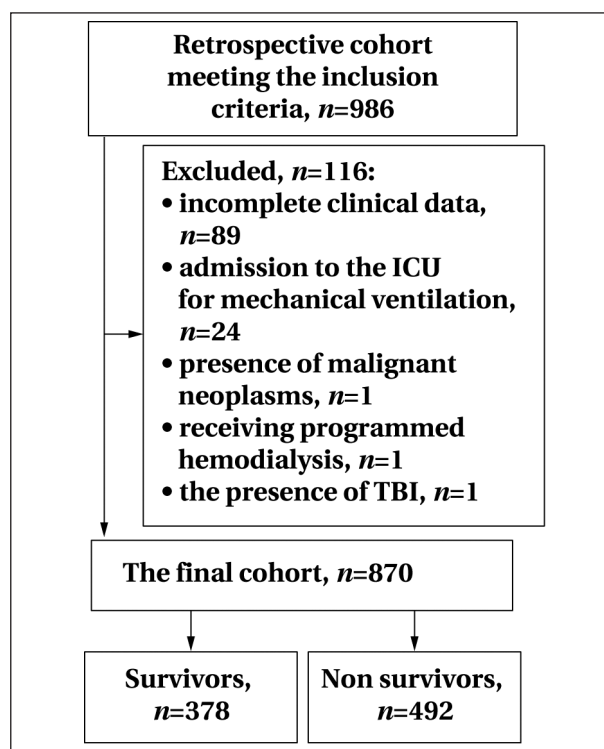


Fig. 1. Patient selection scheme.

length of follow up (FUP) for each patient was defined as the interval from admission to the occurrence of specific event (death, sepsis, or septic shock), or until discharge from the hospital, after which the case was considered as a censored observation. The maximum FUP corresponded to duration of hospital stay for the patient with the longest stay. The achievement of 100% cumulative probability on the Kaplan–Meier curves reflected the fact that by the end of FUP, all patients under FUP had experienced an event, and did not indicate universal mortality or total complication development.

Kaplan–Meier analysis for the REMS scoring system showed statistically significant differences for all events (Table 3, Fig. 3).

For all events, there were statistically significant differences between high-risk and low-risk patients ($p < 0.0001$). The median time to event was statistically significantly shorter in high-risk patients.

Results of univariate Cox regression analysis.

The Cox proportional hazards analysis was also performed only for the REMS.

The results of the analysis (Table 4) showed that REMS demonstrated a statistically significant association for all events: HR 1.215 for death, HR 1.154 for sepsis, and HR 1.214 for septic shock ($p < 0.0001$ in all cases).

In the time-dependent covariate model, the REMS interaction with time did not reach statistical significance for mortality ($p = 0.112$), indicating that there was no significant temporal modification of the effect. The effect decreased over time for sepsis

Table 1. Characteristics of patients upon admission to the ICU/ED.

Parameters	Parameters value			<i>p</i>
	All patients, <i>n</i> =870	Survivors, <i>n</i> =378	Non-survivors, <i>n</i> =492	
Age, y, <i>Me (IQR)</i>	66.0 (55.0–75.0)	57.0 (39.3–68.0)	71.0 (64.0–79.0)	<0.0001*
Mortality, <i>n</i> (%)	492 (56.6)			
Systolic BP, mm Hg <i>Me (IQR)</i>	125.0 (120.0–130.0)	125.0 (120.0–130.0)	126.5 (120.0–131.0)	0.5202
Diastolic BP, mm Hg <i>Me (IQR)</i>	80.0 (70.0–80.0)	79.0 (70.0–80.0)	80.0 (70.0–80.0)	0.9080
Median BP, mm Hg <i>Me (IQR)</i>	93.0 (87.8–97.0)	93.0 (87.0–97.0)	93 (88.0–97.0)	0.5110
HR, bpm, <i>Me (IQR)</i>	82.0 (78.0–90.3)	81.0 (77.0–90.0)	82.0 (78.0–95.0)	0.1974
RR/ min, <i>Me (IQR)</i>	19.0 (18.0–20.0)	18.0 (18.0–20.0)	19.0 (18.0–21.0)	0.0001*
SpO ₂ , %, <i>Me (IQR)</i>	94.0 (88.0–96.0)	95.0 (92.3–97.0)	93.0 (85.0–96.0)	<0.0001*
Body temper., °C, <i>Me (IQR)</i>	37.2 (36.7–37.8)	37.1 (36.7–37.7)	37.2 (36.7–37.8)	0.6112
GCS, scores. <i>Me (IQR)</i>	15 (15–15)	15 (15–15)	15 (14–15)	<0.0001*
Oxygen therapy, <i>n</i> (%)	489 (56.2)	167 (43.3)	322 (66.1)	<0.0001*
Sepsis, <i>n</i> (%)	516 (59.3)	91 (24.1)	425 (86.4)	<0.0001*
Septic shock, <i>n</i> (%)	429 (49.3)	8 (2.1)	421 (85.6)	<0.0001*

Note. BP — blood pressure; HR — heart rate; RR — respiratory rate; GCS — Glasgow Coma Scale. For tables 1, 3: *Me* — median; *IQR* — interquartile range. * — $p < 0.05$ according to the Fisher exact test for qualitative indicators and the Mann–Whitney test for quantitative indicators

Table 2.1. ROC analysis of in-hospital mortality prediction.

Scoring system	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %	Youden J index	<i>p</i>
REMS	0.780 (0.751–0.807)	>4	81.07	61.46	0.4253	<0.0001
NEWS	0.678 (0.646–0.709)	>7	54.12	73.96	0.2807	<0.0001
NEWS2	0.678 (0.646–0.709)	>6	62.96	66.15	0.2911	<0.0001
qSOFA	0.618 (0.584–0.650)	>0	44.03	76.82	0.2086	<0.0001
SIRS	0.578 (0.544–0.611)	>1	41.56	71.09	0.1266	<0.0001

Note. For tables 2.1–2.3: AUC — area under curve; cut-off point with the highest Youden J index value.

Table 2.2. ROC analysis of sepsis prediction

Scoring system	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %	Youden J index	<i>p</i>
REMS	0.677 (0.645–0.708)	>4	73.39	53.63	0.2702	<0.0001
NEWS	0.613 (0.580–0.646)	>5	69.47	47.49	0.1696	<0.0001
NEWS2	0.608 (0.575–0.641)	>6	57.34	60.06	0.1739	<0.0001
qSOFA	0.579 (0.545–0.612)	>0	40.12	72.91	0.1302	<0.0001
SIRS	0.583 (0.549–0.616)	>1	41.68	72.07	0.1375	<0.0001

Table 2.3. ROC analysis of septic shock prediction.

Scoring system	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %	Youden J index	<i>p</i>
REMS	0.724 (0.693–0.754)	>4	79.75	52.90	0.3266	<0.0001
NEWS	0.642 (0.609–0.674)	>7	53.33	68.39	0.2172	<0.0001
NEWS2	0.639 (0.606–0.671)	>6	62.72	60.86	0.2358	<0.0001
qSOFA	0.592 (0.558–0.624)	>1	18.77	96.13	0.1489	<0.0001
SIRS	0.574 (0.541–0.607)	>1	42.22	69.46	0.1168	<0.0001

(HR 0.937 [95% CI 0.904–0.972]) and septic shock (HR 0.961 [95% CI 0.926–0.997]).

Discussion

The analysis confirmed the predictive value of REMS for assessing events in patients with COVID-19, including in-hospital mortality, sepsis, and septic shock. According to the ROC analysis, the REMS scoring system showed the highest AUC for all three endpoints, indicating its best discriminatory capacity in the study cohort. NEWS, NEWS2, qSOFA, and SIRS demonstrated low AUC values, reflecting their limited value for risk stratification in the context of COVID-19.

The results obtained are consistent with previously published studies. In the works of Vedovati et al. [15] and Tsai et al. [16], REMS showed similar values (AUC 0.793 and 0.773, respectively) in predicting mortality. Similar results were obtained by Kamal et al. [17], where a combined endpoint (severe pneumonia, ARDS, sepsis, and septic shock) was evaluated, with an AUC of 0.765, which is close to the data presented in the study.

The NEWS and NEWS2 rapid scoring systems demonstrated low predictive power. This is consistent with the results of J. Alencar et al. [18] and S. Accordino [19], who obtained AUC < 0.7 for NEWS when predicting mortality in patients with COVID-19,

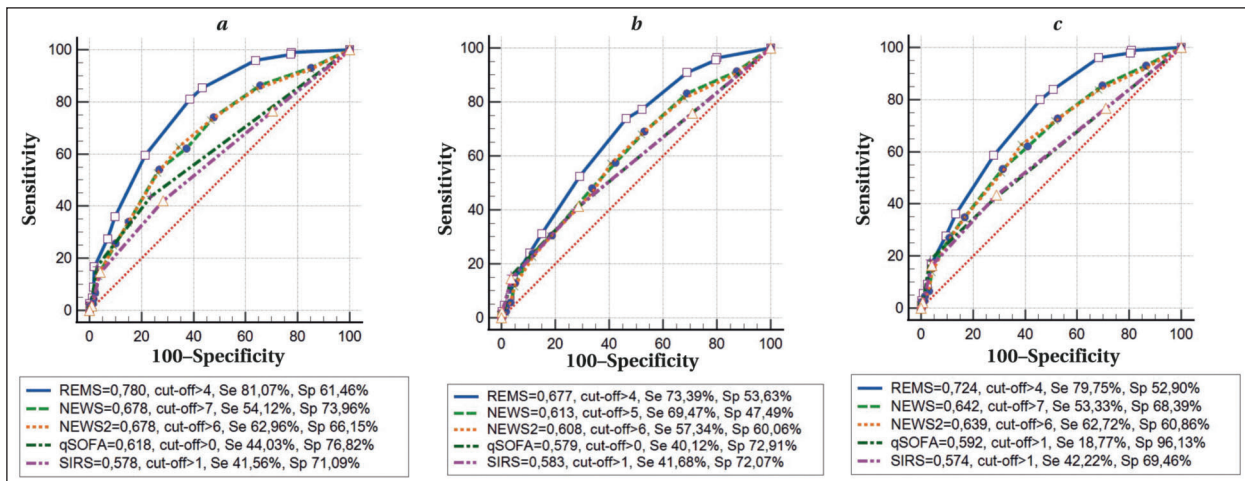


Fig. 2. Comparative ROC curves of the rapid scoring systems in prediction of: (a) death; (b) sepsis; (c) septic shock. Cut-off — is the point with the highest Youden J index; Se — sensitivity; Sp — specificity.

Table 3. Kaplan-Meier analysis of cumulative probability of outcomes (REMS).

Event	χ^2	<i>p</i>	HR (95% CI)	Median time, days, <i>Me</i> (IQR)	
				low risk	high risk
Death	95.50	< 0.0001	2.57 (2.13–3.10)	16 (15–21)	9 (8–10)
Sepsis	45.71	< 0.0001	1.89 (1.57–2.27)	15 (14–16)	10 (9–11)
Septic shock	73.01	< 0.0001	2.42 (1.97–2.96)	18 (16–21)	11 (10–13)

Note. χ^2 is the value of the log-rank test for comparing survival curves; HR is the hazards ratio calculated using the Cox model.

Table 4. Results of univariate Cox regression analysis (REMS).

Outcome	χ^2	<i>p</i>	HR (95% CI)	C (95% CI)
Death	143.69	< 0.0001	1.215 (1.178–1.254)	0.656 (0.630–0.681)
Sepsis	77.75	< 0.0001	1.154 (1.118–1.191)	0.611 (0.585–0.637)
Septic shock	122.72	< 0.0001	1.214 (1.174–1.256)	0.651 (0.624–0.678)

Note. χ^2 is the value of the chi-square criterion for the general model; HR is the hazards ratio; C-index is the Harrell's concordance index; 95% CI is the confidence interval

and the data of L. J. Scott et al. [20] and P.A. G. Ariztizabal et al. [21], who also obtained unsatisfactory results (AUC 0.65 and 0.68, respectively) when evaluating NEWS2 for predicting mortality.

To our knowledge, there are currently no articles with a separate assessment of the predictive power of NEWS and NEWS2 in relation to development of sepsis or septic shock. However, M. Kamal et al. [17] evaluated NEWS2 as a predictor of severe disease (severe pneumonia, ARDS, sepsis, and septic shock), obtaining an AUC of 0.739. However, the sample size was limited ($n=219$), so the results of this study should be interpreted with caution.

For qSOFA and SIRS, the obtained results (AUC 0.58–0.62) also correspond to the published data. J. Alencar et al. [18] reported low accuracy of qSOFA and SIRS in predicting 30-day mortality (AUC 0.55 and 0.58, respectively). In the study by M. Kamal et al. [17] qSOFA AUC equaled 0.671 in prediction of severe disease (severe pneumonia, ARDS, sepsis, and septic shock),

For the first time, the REMS was evaluated as a risk stratification tool in the analysis of time to adverse outcome in patients with COVID-19. It was found that stratification of patients into low (≤ 4 scores) and high (> 4 scores) risk groups using

REMS scoring system was associated with statistically significant differences in the time to adverse events, which was confirmed using the Kaplan–Meier method.

Unlike previously published studies [22], which mainly applied survival analysis methods to individual physiological parameters included in the REMS scoring system, the present study used the total REMS score as an integral prognostic indicator in the TTE analysis.

The Kaplan–Meier analysis data were consistent with the Cox regression analysis, which showed that REMS had the highest hazard ratio (HR=1.215; 95% CI 1.178–1.254) and the highest concordance index (C=0.656). This confirms the steady relationship between the severity of illness expressed by REMS and the probability of adverse events. However, in the analysis of time to death, sepsis and septic shock, the proportional hazards assumption for the REMS was violated. To correctly assess the effect of REMS, a Cox model with a time-dependent covariate was used, taking into account that REMS influence on the risk evolves over time. The prognostic value of REMS for sepsis and septic shock was highest in the early stages of hospital stay and gradually decreased over time, highlighting its high clinical sig-

nificance for early identification of patients at high risk of adverse events. For mortality, the temporal dependence of the REMS effect did not reach statistical significance.

It should be noted that the results of this retrospective study largely agree with the results of earlier published by our group meta-analysis [23], where the REMS, NEWS, NEWS2, qSOFA rapid scoring systems and the SIRS criteria showed similar performance in predicting in-hospital mortality (AUC 0.808; 0.782; 0.749; 0.722 and 0.662, respectively) and adverse events (AUC 0.733; 0.778; 0.778, 0.662, and 0.607, respectively), however, the quality of GRADE evidence for all rapid scoring systems was low or very low due to high risks of systematic error, possible publication bias, or high heterogeneity. However, only the REMS scoring system had moderate evidence quality due to its strong effect (AUC for mortality of 0.82), low risk of bias, and lack of publication bias.

The advantage of REMS is probably due to its comprehensive nature: the scoring system takes into account not only physiological parameters (respiratory rate, heart rate, blood pressure, level of consciousness and saturation), but also age — an independent predictor of mortality in COVID-19. Thus, REMS reflects both the severity of the systemic inflammatory response and the reserve capacity of the body.

The unsatisfactory predictive power of NEWS, NEWS2, qSOFA, and SIRS is likely due to deviation from their intended use. NEWS and NEWS2 were primarily designed for early detection of disease worsening in hospitalized patients, while qSOFA and SIRS are focused on screening for sepsis rather than predicting outcomes in critically ill patients. It should be mentioned that the assessment of the qSOFA prognostic significance for identifying patients at higher risk of developing or already experiencing sepsis or septic shock has a methodological limitation associated with the partial overlap of the qSOFA predictors with the clinical criteria used to establish these complications, which may lead to biased estimates of prognostic accuracy. Despite this limitation, the analysis of the qSOFA predictive power for sepsis and septic shock appears to be clinically relevant, as this RSS was initially proposed as a tool for early identification of patients with infection who are at increased risk of adverse outcomes.

It is important to note that in the current study, the prognostic characteristics of NEWS, NEWS2, qSOFA, and SIRS were lower than in several publications from the early stages of the COVID-19 pandemic and in our previous meta-analysis [23]. This may be due to several factors. Firstly, the study included only a cohort of ED patients with high incidence of sepsis, septic shock, and high mortality rate, while numerous early studies analyzed more

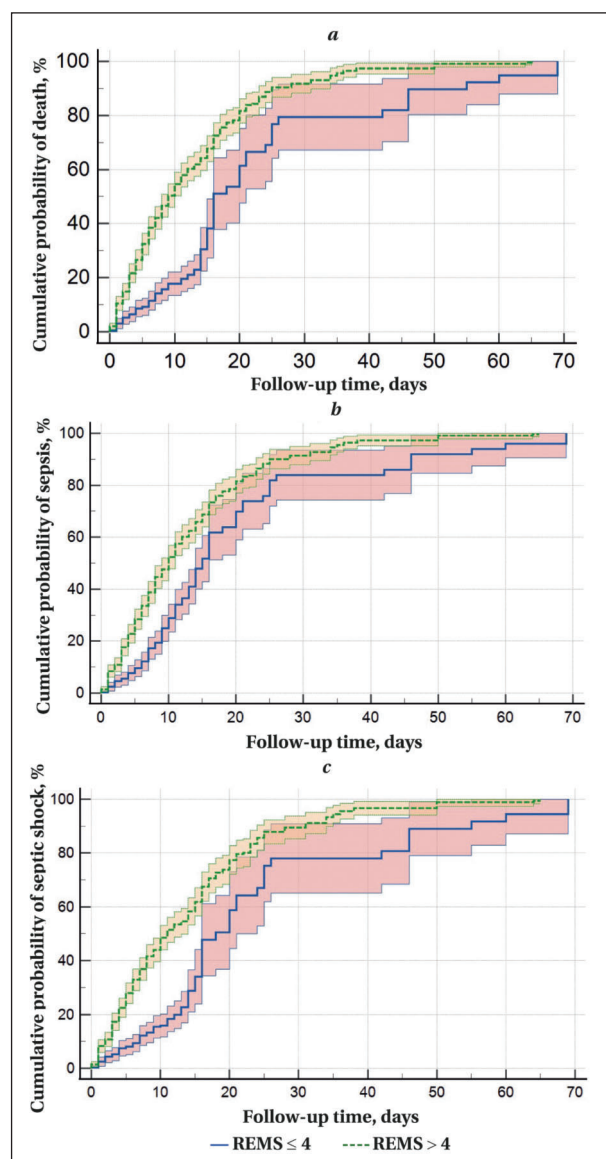


Fig. 3. Kaplan-Meier curves for mortality (a); sepsis (b); and septic shock (c).

heterogeneous hospital cohorts. In the cohort of initially severely ill and clinically homogeneous patient population, the discriminatory capacity of universal rapid scoring systems naturally decreases. Secondly, studies in the early stages of COVID-19 pandemic were conducted in different clinical and epidemiological situation, with different structure of hospitalized patients, different treatment strategies, and a lack of experience in managing this patient population, making it difficult to directly compare AUC values. Thirdly, some of the early publications reported combined endpoints that included admission to the intensive care unit, respiratory failure, mechanical ventilation, or other adverse events, whereas the current study focused on specific adverse clinical events. Finally, some of the high scores reported in earlier studies should be interpreted with caution, as the quality of evidence for most of the RSS remained low or very low due to

significant heterogeneity, the risk of bias, and potential publication bias [23].

It is worth noting that the inclusion of the NEWS, NEWS2, qSOFA scoring systems and SIRS in the analysis of mortality prediction was due to their widespread use in real clinical practice as rapid tools for initial assessment of disease severity and risk stratification. During the pandemic, these RSS were often used at the pre-hospital stage, in admission departments, and for triage, making it clinically relevant to evaluate their predictive capacity in relation to the most severe endpoint, which is mortality. Thus, inclusion of these scoring systems in the study had a comparative goal of their performance evaluation, i.e., determining the extent to which widely used rapid scoring tools retain their discriminatory capacity in a cohort of severely ill COVID-19 patients admitted to ICU/ ED.

The results obtained are consistent with the current data that REMS is a universal tool capable of assessing the risk of death and complications in a wide range of critically ill patients. The use of REMS in clinical practice can improve the accuracy of early risk stratification in patients with COVID-19 and facilitate the timely initiation of intensive care.

The study has several limitations. Its retrospective nature may contribute to systematic errors in data collection, and its single-center design limits the generalizability of the results. Additionally, there is a lack of external validation, which requires confirmation of the findings in independent samples. Nevertheless, the use of three independent statistical approaches (ROC analysis, survival analysis, and Cox regression) ensured high consistency and sta-

tistical significance of the obtained data. It is important to note that the study sample consisted of ICU/ ED patients, yielding high incidence of fatal outcomes, sepsis, and septic shock. Therefore, the results obtained characterize the predictive value of the studied rapid scoring systems in the population of hospitalized severely ill patients, and cannot be directly extrapolated to a broader population of patients with COVID-19.

In general, the results confirm that the REMS is the most accurate tool for predicting in-hospital mortality and septic complications in patients with COVID-19, while NEWS and NEWS2 are suitable for dynamic monitoring, and qSOFA and SIRS can be used as auxiliary screening tools at the pre-hospital and early hospital stages.

Conclusion

The REMS, NEWS, NEWS2, qSOFA rapid scoring systems, and SIRS have a statistically significant value for prediction of sepsis, septic shock and in-hospital mortality in patients with COVID-19.

Only the REMS scoring system showed satisfactory accuracy for predicting mortality and septic shock in patients with COVID-19.

The NEWS, NEWS2, qSOFA scoring systems, and SIRS have unsatisfactory predictive power, and cannot be recommended for stratifying the severity of illness in patients with COVID-19, and for predicting adverse events when used as tools for primary risk stratification upon admission. However, these scoring systems are valuable for dynamic monitoring of patients' condition.

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Comparative Assessment of Equivalence of the Post-Operative Use of Two Amino Acid Solutions

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Summary

Objective: to compare the nutritional and metabolic effects of two amino acids-containing solutions, differed in amino acid profiles and sorbitol presence, when employed as a part of standardized combined post-operative nutritional support in patients undergoing surgery for abdominal cancer.

Materials and Methods. A prospective randomized study was conducted in two specialized hospitals. The study included 120 nutritionally stable patients aged 18–80 with stage I–III colorectal tumors and ASA class II–III anesthetic risk (mean BMI 25.3–27.6 kg/m²), who underwent elective abdominal surgeries and required parenteral administration of protein-carbohydrate nutrition solution (PCNS) for at least 5 days. Patients were randomly assigned into two groups for receiving PCNS formulations Amiparcenin or Aminoven formulated at 10-percent concentration (60 patients, each group). Both regimens involved administering an amino acid solution at 10 ml/kg/day in combination with a 10% dextrose solution at 10 ml/kg/day, alongside with early enteral feeding. The amino acid and nutritional profiles were assessed before initiation of nutritional support (T0), on the 2nd (T1), and 5th (T2) postoperative days. Key indicators included the sum of branched-chain amino acids (BCAA_sum), the sum of aromatic amino acids (AAA_sum), the ratio of BCAA/AAA, the integral indicator of amino acids in the urea cycle (UreaCycle_sum), and index ratios of ornithine to citrulline, arginine to ornithine, and arginine to citrulline. Additionally, total protein, albumin, nitrogen balance, bioimpedance indicator of active cellular mass (BCM), creatinine, liver function markers, and electrolyte profiles were analyzed. To evaluate clinical equivalence, a two one-sided tests (TOST) analysis was employed with pre-defined limits of clinically acceptable difference (Δ) for each parameter.

Results. By day 5, both groups showed statistically significant changes in total protein and albumin levels, reflecting the transition from the early catabolic phase and the impact of nutritional support; no intergroup differences were observed for these parameters, and the TOST analysis confirmed their equivalence within the predefined limits of Δ . The branched-chain amino acids (BCAA_sum) level increased from T0 to T1 and then stabilized at T2 in both groups; the factors «group» and the «group×period» interaction were not significant ($p=0.285$ and $p=0.362$, respectively), however, the equivalence of absolute BCAA_sum values at T2 with a narrow $\Delta=10\text{ }\mu\text{mol/L}$ was not confirmed ($p\text{-TOST}>0.05$). For AAA_sum, differences were documented based on the «group» factor ($p=0.035$) and the interaction «period×group» ($p=0.0099$), with alignment of absolute values at T2. The ratio of BCAA/AAA and the index indicators of the urea cycle (Orn/Cit, Arg/Orn, Arg/Cit) remained stable, with no intergroup differences, and met the equivalence criteria throughout all observation periods. According to bio-impedance analysis, the Amiparcenin group showed a more significant increase in active cell mass (p by Friedman = 0.00001; intergroup p at T1 = 0.0048, at T2 = 0.00014), while the Aminoven group did not show significant dynamics; moreover, the TOST analysis with a wide Δ showed formal equivalence in BCM. Creatinine, urea, transaminases, and electrolyte levels remained comparable between the groups and were within clinically acceptable ranges; equivalence for these parameters was confirmed.

Conclusion. In the early postoperative period, Amiparcenin and Aminoven 10% solutions provide comparable amino acid and nutritional supply, and biochemical effects as a part of standardized combined nutritional support in patients with abdominal cancers. The studied regimens met equivalence criteria for most essential amino acid indicators (including BCAA/AAA), markers of inflammation, protein metabolism, liver and kidney function, and electrolyte balance. The differences found in absolute values of BCAA_sum, AAA_sum, UreaCycle_sum, and malondialdehyde (MDA) do not translate into clinically significant shifts during the first 5 days post-surgery.

Keywords: *amiparcenin; aminoven; parenteral nutritional support; amino acid solutions; abdominal cancer surgery; nitrogen balance; amino acid profile*

Conflicts of Interest. The authors declare no conflicts of interest.

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Introduction

For most patients with gastrointestinal neoplasms, nutritional deficiency and cachexia at the time of hospitalization are not a theoretical risk but a reality. According to international and national studies, 30–70% of cancer patients experience weight loss, muscle tissue loss, and metabolic disorders, which are associated with an increased incidence of postoperative complications, prolonged hospital stays, and worsened prognosis [1–4].

ESPEN guidelines on nutrition for cancer patients, clinical nutrition in surgery, ESMO clinical recommendations, and domestic documents emphasize that assessing nutritional status and its correction should be considered a mandatory part of the standard management of patients with gastrointestinal neoplasms [1–3, 5–9]. They highlight the necessity of early initiation of nutrition, prioritizing the enteral route while adding a parenteral component when it is not possible to meet the target protein and energy intake solely through the gastrointestinal tract.

A combined approach (enteral + parenteral nutrition) is considered a rational strategy when it is expected that enteral nutrition alone in the initial days will not cover ≥ 50 –60% of protein and energy needs, or in the presence of high nutritional risk [5–9]. In such situations, the parenteral component provides the opportunity to «catch up» on amino acids and calories while the enteral route is limited.

A key component of the protocol parenteral part is the amino acid solution. It determines the specific set of amino acids that the patient actually receives, how the amino acid profile of the plasma changes, and how effectively the body can compensate for surgical stress. The composition of standard and specialized mixtures (including nitrogen content, the ratio of branched-chain and aromatic amino acids, the presence of arginine, histidine, sulfur-containing amino acids, etc.) and their potential impact on outcomes are discussed in detail in the ESPEN reviews and guidelines on parenteral nutrition and perioperative management [10–13].

A particular significance is given to the Fisher ratio — the ratio of branched-chain amino acids to aromatic amino acids (BCAA/AAA). It has been shown that a decrease in this ratio in liver cirrhosis is associated with the risk of hepatic encephalopathy and an unfavorable prognosis, while its normalization is considered a desirable outcome of nutritional therapy [14, 15]. In this regard, BCAA-enriched solutions are being actively introduced for patients with severe liver failure; however, for surgical patients without decompensated liver function, standard amino acid mixtures remain the cornerstone of therapy [5, 10–15].

Despite the large amount of data on nutrition for cancer patients in general, the question of how the choice of a specific standard amino acid solution affects the amino acid profile in plasma and nitrogen metabolism in surgical patients has been studied only to a limited extent. There are individual randomized studies that compared different amino acid compositions in critically ill patients (neurosurgery, bone marrow transplantation, neonatology) with an assessment of the amino acid profile, nitrogen balance, and clinical parameters [12–19]. However, most of these studies either focus on specialized formulas (BCAA or glutamine-enriched solutions) or are dedicated to comparing routes of administration and conventional protocols (enteral vs. parenteral, premixes vs. compounded mixtures) [5, 12, 13, 19–21]. Direct studies on comparability of the clinical and biochemical effects of standard amino acid solutions in the context of strictly standardized combined nutritional support for surgical cancer patients are virtually nonexistent.

An additional issue is that in clinical practice, various standard amino acid solutions, differing in the number of amino acids, their ratios, and the presence or absence of non-protein calories (such as sorbitol), are often used interchangeably as components of parenteral nutrition [10–12, 22]. However, the evidence base supporting their equivalence in key amino acid and nutritional parameters is extremely limited. Modern reviews emphasize that the choice of amino acid formula for parenteral nutrition (PN) is largely based on theoretical consid-

rations and collateral data, with few well-designed equivalence (or non-inferiority) trials of different standard solutions conducted [14, 15, 21, 23].

In this regard, it is relevant to conduct a study that does not claim pharmacokinetic bioequivalence of two formulations, but rather assesses the equivalence of the clinical and biochemical effects of two standard amino acid solutions that differ in composition and the presence of an additional energy substrate, under conditions of strictly standardized combined nutritional support in a homogeneous group of patients with colorectal neoplasms.

The aim of this study is to evaluate, based on key amino acid, biochemical, and bioimpedance indicators, how comparable the nutritional and metabolic effects of the two amino acid solutions, which differ in their amino acid profiles and the presence of sorbitol, are when used as part of standardized combined postoperative nutritional support in patients undergoing surgery for abdominal cancer.

Materials and Methods

Study Design. A prospective, randomized, controlled study was conducted to assess the clinical and biochemical comparability of two amino acid solutions for parenteral nutrition. The study involved two specialized hospitals located in Saint Petersburg and Nizhny Novgorod. The enrollment period was from July to December 2025.

The study protocol was approved by the local ethics committee of the L.G. Sokolov Northwest District Scientific Clinical Center of the Federal Medical-Biological Agency of Russia (protocol No. 4 dated May 22, 2025) and was agreed upon at the inter-center level. The study complied with the principles of the Helsinki Declaration and Good Clinical Practice (GCP) guidelines. Written informed consent was obtained from all patients. The study protocol was not registered in publicly accessible clinical trial registries, which was considered a methodological limitation.

After screening and signing the informed consent, patients were randomized into two groups based on the assignment of either Aminoven 10% or Amiparcenin.

- Aminoven group (the control group);
- Amiparcenin group (the study group).

The ratio was 1:1. The random allocation sequence was generated by an independent statistician using a computer-generated list. To implement the randomization, sealed opaque numbered envelopes were used, which were opened only after the patient was finally included and provided informed consent.

The sample size (a minimum of 120 patients, with 60 in each group) was calculated in advance based on the primary endpoint — the BCAA/AAA ratio on the 5th day after surgery (T2). The planning assumed an expected mean BCAA/AAA ratio of

around 3.0 in both groups, a standard deviation of approximately 0.5, and a clinically acceptable difference interval $\Delta = \pm 0.3$ (about $\pm 10\%$ of the mean). With a significance level of $\alpha = 0.05$ and a power of $1 - \beta = 0.90$, the required sample size was at least 54 patients in each group; considering possible dropout from the per-protocol population, the target size was increased to 60 patients per group (a total of 120 patients).

The flowchart of patient screening, randomization, follow up, and inclusion in various analysis populations is presented in Fig. 1.

Inclusion Criteria. Patients were included if they met all of the following criteria:

1. Age between 18 and 80 years.
2. Confirmed stage I–III colorectal neoplasm.
3. Elective radical or debulking surgery on the gastrointestinal tract.
4. Anesthetic risk classified as II–III according to the American Society of Anesthesiologists (ASA) scale.
5. Surgical anesthetic risk according to the P POSSUM scale greater than 5%.
6. Expected duration of combined nutritional support of at least 5 days due to the inability or insufficient effectiveness of purely enteral nutrition (in accordance with current clinical guidelines for perioperative nutritional support [1–3, 5–11]).
7. Signed informed consent.

Exclusion criteria. Patients were excluded from the study or from the per protocol analysis if they had:

- refractory cachexia (9–12 scores on the 2018 cachexia staging scale [2]);
- stage IV cancer;
- subcompensated or decompensated diabetes mellitus;
- significantly reduced glomerular filtration rate (< 60 mL/min);
- liver failure in a sub- or decompensated stage;
- congestive heart failure classified as NYHA functional class III–IV;
- implanted pacemaker;
- refusal to participate in the study.

During the follow-up period, patients were excluded from the per protocol analysis if they developed:

- pulmonary embolism, acute myocardial infarction, or acute cerebrovascular accident;
- acute gastrointestinal bleeding;
- pneumothorax;
- acute kidney injury;
- severe infectious complications occurring within the first 5 days post-surgery.

Randomized patients who initiated treatment but were excluded for the specified reasons were retained in the ITT population but were not included in the PP analysis.

Nutritional support and the intervention under investigation. All patients received combined nutritional support—a combination of enteral and parenteral nutrition—in accordance with the recommendations of the European Society for Clinical Nutrition and Metabolism and domestic clinical guidelines [1–3, 5–11]. The parenteral component was considered an adjunct to early enteral nutrition rather than as total parenteral nutrition.

Calculation of nutritional needs. Ideal body weight was calculated using the Broca formula (weight, kg = height, cm – 100). Daily requirement for amino acids: 1.2 g/kg of ideal body weight. Daily energy requirement: 25 kcal/kg of ideal body weight. Contribution of protein: 4 kcal per 1 g of amino acids.

The remaining energy was distributed between carbohydrates and fats, adhering to recommended infusion rates (for glucose — no more than 0.5 g/kg per hour, for fat emulsions — no more than 0.11 g/kg per hour) [1–3, 5–11].

Parenteral component. In the Amiparcenin group, patients received an amino acid solution (11 amino acids, amino acid concentration about 9.1%, additional energy source — sorbitol 50 g/L, = 200 kcal/L) at a dose of 10 ml/kg/day (averaging 500–700 ml/day), which corresponded to approximately 1.0–1.1 g of amino acids/kg/day. The solution was primarily administered through a central venous catheter at a rate not exceeding 1 ml/min, simultaneously with a 10% dextrose solution at 10 ml/kg/day.

In the Aminoven 10% group, patients received a standard amino acid solution (15 amino acids, amino acid concentration 10%, without additional non-protein calories) at a dose of 10 ml/kg/day (also = 1.0–1.1 g of amino acids/kg/day) and a 10% dextrose solution at 10 ml/kg/day. Additional non-protein calories in both groups were primarily provided through enteral nutrition; when necessary, fat emulsions could be used at the discretion of the attending physician, but they were not the subject of comparative analysis in this study. This means that the use, dosage, and type of fat emulsions were not controlled by the protocol, which may be considered an additional uncontrolled factor that could affect energy balance, nitrogen metabolism, and dynamics of body cell mass.

Enteral component. Enteral nutrition (via nasointestinal tube) was generally initiated on the following day after surgery. The volume, concentration, and daily dose of the standard nutritional mixture were gradually increased until at least 70% of the calculated daily protein and energy requirements were met [1–3, 5–11].

Assessment of effectiveness. The following indicators were evaluated:

1. Amino acid profile:

- Total branched-chain amino acids (BCAA_sum: leucine, isoleucine, valine);

- Total aromatic amino acids (AAA_sum: phenylalanine, tyrosine, tryptophan);
- Ratio of BCAA to AAA (Fisher's ratio) [14,15];
- Indicators of the urea cycle amino acids: ornithine, citrulline, arginine; composite indicator UreaCycle_sum and index ratios of ornithine/citrulline, arginine/ornithine, arginine/citrulline.

These indices are traditionally used in cases of liver failure, but we considered them more broadly—as integral markers of amino acid balance and nitrogen metabolism, applicable in surgical patients without severe liver decompensation [14–16].

2. Nutritional status and body composition:

- Bioelectrical impedance analysis (DIAMANT device, Russia): body cell mass (BCM).

Among the various parameters of bioelectrical impedance analysis, we pre-selected body cell mass as the primary bioelectrical impedance endpoint, as BCM is most consistently associated with the risk of complications and survival in cancer patients [4,24]. This allowed us to prioritize the analysis and avoid excessive multiplicity of comparisons.

3. Laboratory and clinical parameters:

- total protein, albumin;
- creatinine, cystatin C;
- AST, ALT, De Ritis ratio, bilirubin;
- C-reactive protein;
- nitrogen balance based on daily nitrogen output with urea.

All parameters were collected at three reference time points: before initiation of nutritional support (T0), on the second (T1), and fifth (T2) postoperative days.

Primary endpoints:

1. BCAA_sum;
2. AAA_sum;
3. BCAA/AAA ratio;
4. C-reactive protein (CRP) concentration.

The primary endpoint selected for sample size calculation and formal equivalence analysis is the BCAA/AAA ratio at day 5 (T2).

Secondary endpoints: protein metabolism indicators (total protein, albumin), BCM, nitrogen balance, liver and kidney function markers, electrolytes, and composite and index indicators of the urea cycle.

Populations for analysis:

- Intention to treat (ITT): all 120 randomized patients were analyzed according to the initial randomization.
- Per protocol (PP): patients who completed the protocol without significant deviations and received the parenteral component of combined nutritional support for at least 5 days.

Equivalence and changes in indicators were primarily analyzed in the PP population; the ITT analysis was used to verify the robustness of the results and to reflect «real-world clinical practice».

Laboratory and instrumental methods used.

The plasma amino acid profile was determined

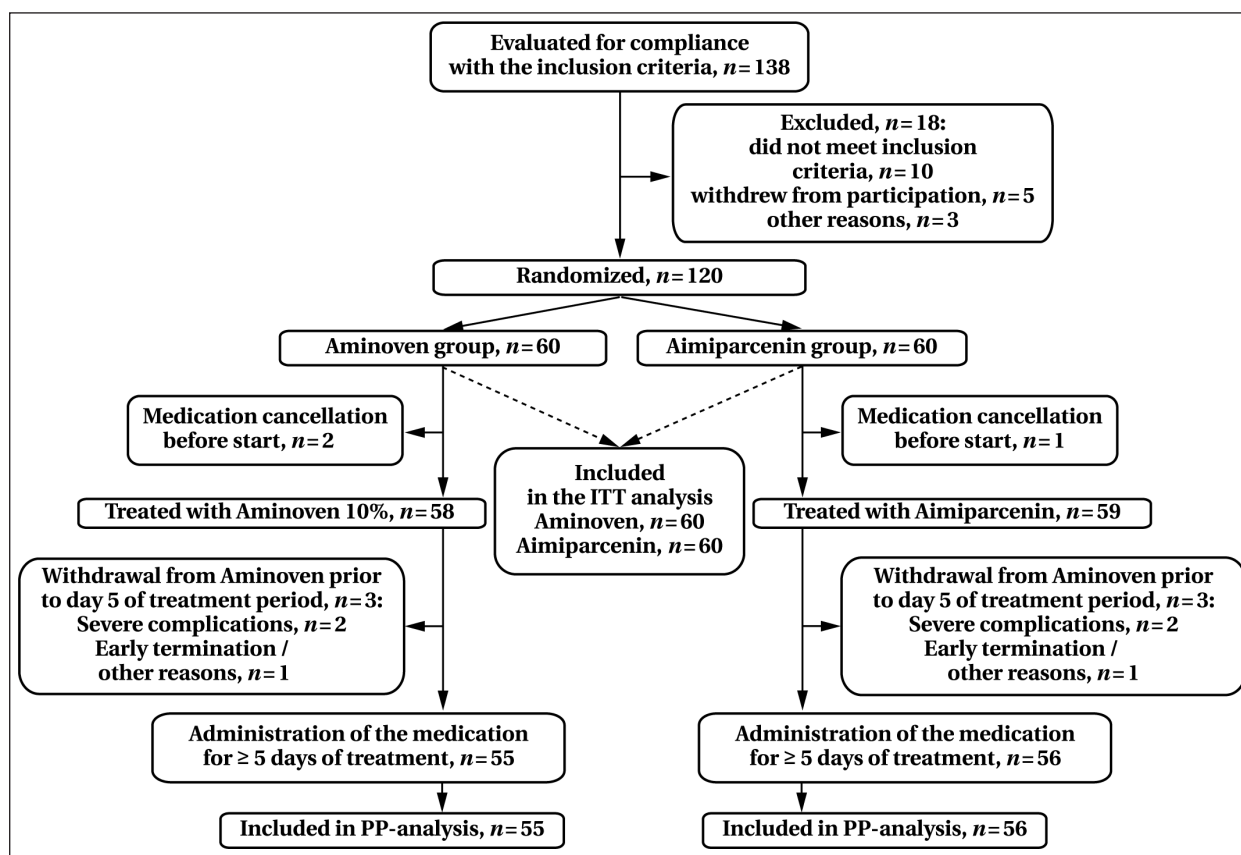


Fig. 1. Scheme for selecting and including patients in the study.

using high-performance liquid chromatography (Agilent 1260 Infinity analyzer, USA). Biochemical parameters (total protein, albumin, creatinine, urea, AST, ALT, bilirubin, CRP, electrolytes) were analyzed on the automated Cobas c702 analyzer (Roche). Cystatin C was measured by immunonephelometry (BN ProSpec, Siemens). Malondialdehyde (MDA) was assessed using a spectrophotometric method with thiobarbituric acid. Body cell mass was evaluated by bioimpedance analysis (DIAMANT, Russia). All methods are validated, with variability $\leq 8\%$.

Statistical analysis. We used Statistica version 15 (StatSoft, USA). The normality of the distribution was assessed using the Kolmogorov–Smirnov tests (with Lilliefors correction) and the Shapiro–Wilk test. Normally distributed data were presented as $M \pm SD$ with a 95% confidence interval; non-normally distributed data were presented as $Me [Q1; Q3]$. To compare independent groups, we used the Student's *t*-test or the Mann–Whitney *U* test; for paired measurements, we used the paired *t*-test or the Wilcoxon test.

For analyzing changes over time (T0, T1, T2), we applied repeated measures ANOVA or the Friedman test. When there was overall significance, we conducted pairwise comparisons between the reference time points using the paired *t*-test or the Wilcoxon test with the Holm–Bonferroni correction for multiple comparisons.

The influence of the «group» and «period» factors and their interaction («group×period») on BCAA_sum and AAA_sum indicators, the BCAA/AAA ratio, and the level of C-reactive protein was assessed using repeated measures ANOVA, calculating the partial eta squared (partial η^2).

To account for potential confounding factors (age, sex, baseline biochemical parameters), additional correction was performed in several models (ANCOVA/repeated measures models including covariates).

Analysis of equivalence (TOST). To assess the equivalence between groups regarding BCAA/AAA, index parameters of the urea cycle, nitrogen balance, creatinine, total protein, albumin, liver function tests, and electrolytes, a Two One-Sided Tests (TOST) approach was employed. For each parameter, a clinically acceptable range of difference $[-\Delta; +\Delta]$ (zone of clinical insignificance) was established prior to the study. Equivalence was considered established if:

- The 90% confidence interval of the mean difference (or the ratio of geometric means for log-transformed parameters) was entirely within the range $[-\Delta; +\Delta]$;

- Both one-sided TOST tests had $p < 0.05$.

For the composite amino acid parameters (BCAA_sum, AAA_sum, UreaCycle_sum), relative Δ expressed as percentages were used, while for

electrolytes, absolute Δ were applied ($\text{Na}^+ \pm 3 \text{ mmol/L}$, $\text{K}^+ \pm 0.3 \text{ mmol/L}$, $\text{Cl}^- \pm 3 \text{ mmol/L}$, $\text{Ca}^{2+} \pm 0.1\text{--}0.15 \text{ mmol/L}$). The results of the TOST analysis were compiled into a separate table (Table 3), indicating the effect, 90% CI, and the conclusion of «equivalent / not equivalent».

Missing data. Missing values for key indicators and reference time points were sporadic. ANOVA and the Friedman test were used only for patients with a complete set of data for the corresponding indicator. Multiple imputation was not applied. The potential impact of missing data was addressed in the «Study Limitations» section.

Differences were considered statistically significant at $p < 0.05$.

Results

Patients' baseline characteristics. A total of 120 patients were included, with 60 individuals in each group. Patients' nutritional status was preserved with average BMI in the Amiparcenin group of $27.64 \pm 3.59 \text{ kg/m}^2$, and in the Aminoven group — of $25.34 \pm 3.84 \text{ kg/m}^2$, signifying overweight. No significant changes in biochemistry panel parameters were recorded at baseline. The Amiparcenin and Aminoven groups were comparable in terms of age, sex, tumor location and stage, anesthetic risk level, as well as the extent and duration of surgery (Table 1).

At baseline (T0), the measurements of body weight, body mass index, bioimpedance parameters (including body cell mass), total protein levels, albumin, creatinine, and liver function showed no clinically significant differences between the groups (Table 1).

Based on the amino acid profile at baseline, no differences were found in the total amount of

branched-chain amino acids (BCAAs) and the ratio of BCAA to aromatic amino acids (AAA); the total AAA in the Amiparcenin group was lower than in the Aminoven group, which subsequently affected the dynamics of this indicator (Table 2).

Amino acid profile. The overall dynamics of relative increases in amino acids plasma levels following the administration of various amino acid solutions are presented in heat maps (Fig. 2) and summary graphs (Fig. 3). This indicates a high degree of comparability of the amino acid effect between two regimens.

The total of branched-chain amino acids (BCAA_sum, Fig. 3, a). Following the initiation of combined nutritional support, BCAA_sum increased in both groups by T1, with a slight decrease or stabilization by T2, indicating a consistent trend in both groups.

Within-group analysis (Friedman test):

- Aminoven: $p = 0.2285$;
- Amiparcenin: $p = 0.0515$.

Inter-group differences in BCAA_sum at each reference time-point were not statistically significant (T0: $p = 0.110$; T1: $p = 0.379$; T2: $p = 0.740$). Repeated measures ANOVA confirmed the effect of time ($p = 0.0209$) with no significant «group» or «group $\times$ period» interaction effects.

The TOST analysis with a strict equivalence interval ($\Delta = 10 \mu\text{mol/L}$) did not confirm two regimens equivalence in terms of absolute BCAA_sum values at T2, despite the similar trends observed.

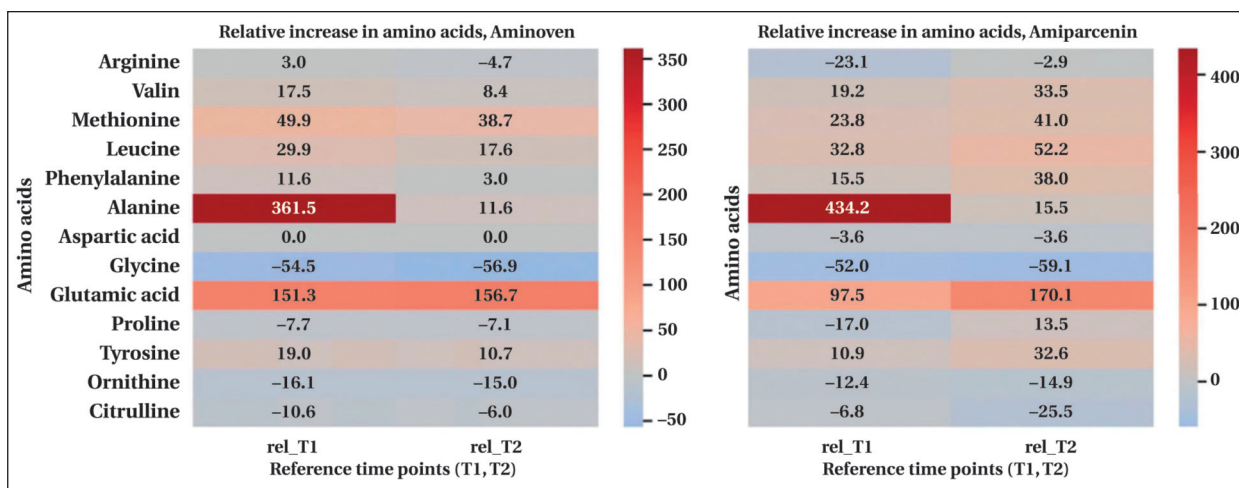
The total of aromatic amino acids (AAA_sum, Fig. 3, b). The dynamics of AAA_sum varied across groups. In the Amiparcenin group, a pronounced overall dynamic was recorded according to the Friedman test ($p = 0.00188$), showing a trend towards

Table 1. Patients' baseline characteristics.

Parameters	Parameter values in the groups		<i>p</i>
	Amiparcenin, <i>n</i> = 60	Aminoven, <i>n</i> = 60	
Age, years	66.8 $\pm$ 6.4	60.6 $\pm$ 13.8	0.002
Males, <i>n</i> (%)	37 (61.7)	24 (40.0)	0.028
BMI	27.64 $\pm$ 3.59	25.34 $\pm$ 3.84	0.34
Neoplastic site: benign tumor of the colon, <i>n</i> (%)	4 (6.7)	0 (0.0)	0.115
Neoplastic site: sigmoid and rectum, <i>n</i> (%)	14 (23.3)	13 (21.7)	0.115
Neoplastic site: colon (proximal), <i>n</i> (%)	42 (70.0)	47 (78.3)	0.115
Stage: I	19 (31.7)	19 (31.7)	0.976
Stage: II	22 (36.7)	23 (38.3)	0.976
Stage: III	19 (31.7)	18 (30.0)	0.976
ASA class: II	40 (66.7)	38 (63.3)	0.848
ASA class: III	20 (33.3)	22 (36.7)	0.848
Duration of surgery, min	195.6 $\pm$ 30.4	185.8 $\pm$ 42.4	0.151
Epidural analgesia, <i>n</i> (%)	42 (70.0)	40 (66.7)	0.844
Body cell mass (BCM) T0, kg	47.43 $\pm$ 2.88	48.05 $\pm$ 4.47	0.371
Total protein, g/L	56.77 $\pm$ 8.45	59.80 $\pm$ 5.35	0.221
Albumin, g/L	33.12 $\pm$ 5.32	31.80 $\pm$ 3.52	0.113
Creatinine, $\mu\text{mol/L}$	92.45 $\pm$ 29.26	102.83 $\pm$ 33.19	0.072
AST, U./L	32.38 $\pm$ 12.05	35.53 $\pm$ 17.92	0.261
ALT, U./L	37.27 $\pm$ 10.62	40.73 $\pm$ 17.47	0.192
Bilirubin, $\mu\text{mol/L}$	10.57 $\pm$ 4.55	16.04 $\pm$ 7.70	0.000
De Ritis Ratio, CU	0.87 $\pm$ 0.28	0.92 $\pm$ 0.34	0.371

Table 2. Baseline total amino acid values (T0) and p-values of intergroup differences (Mann–Whitney test).

Parameters	Parameter values in the groups, $M \pm SD$		p (T0)
	Aminoven	Amiparcenin	
BCAA_sum	384.8 $\pm$ 103.7	327.0 $\pm$ 108.8	0.1094
AAA_sum	138.3 $\pm$ 42.0	103.0 $\pm$ 31.2	0.208
BCAA_AAA_ratio	2.9 $\pm$ 0.9	3.3 $\pm$ 0.8	0.2599
UreaCycle_sum	139.7 $\pm$ 35.8	152.3 $\pm$ 67.3	0.9527

**Fig. 2. Heat maps of average relative increases in amino acids (% from T0) in the Aminoven and Amiparcenin groups.**

an increase at T1 and a significant rise at T2 compared to T0 and T1. In the Aminoven group, changes in AAA_sum were less obvious and did not reach statistical significance ($p=0.09697$).

Intergroup comparisons showed:

- At T0, AAA_sum was lower in the Amiparcenin group ($p=0.019$);
- At T1, the differences persisted ($p=0.006$);
- By T2, the values were closer together, and no statistically significant difference was observed ($p=0.338$).

Repeated measures ANOVA revealed a significant effect of the «group» and «period» factors and their interaction, reflecting different trajectories of AAA_sum changes, followed by the alignment of absolute values by the fifth day. The TOST analysis did not confirm the equivalence of absolute AAA_sum levels at early stages, but by T2, the differences diminished and were not accompanied by noticeable clinical effects (Table 3).

The ratio of branched-chain and aromatic amino acids (BCAA/AAA, Fig. 3, c). The BCAA/AAA

Table 3. Results of the Amiparcenin and Aminoven groups equivalence analysis (TOST) (PP population, $n=120$).

Parameters	Period	Δ^*	Conclusion
BCAA_sum	T2	$\pm 10 \mu\text{mol/L}$	Not confirmed
AAA_sum	T0, T1, T2	$\pm 15\%$	Not confirmed
BCAA/AAA ratio	T0, T1, T2	$\pm 15\%$	Confirmed
UreaCycle_sum	T2	$\pm 15\%$	Not confirmed
Orn/Cit, Arg/Orn, Arg/Cit	T2	$\pm 15\%$	Confirmed
C-reactive protein (CRP), GMR	T0, T1, T2	$\pm 25\%$	Confirmed
Total protein	T2	$\pm 10\%$	Confirmed
Albumin	T0, T1, T2	$\pm 10\%$	Confirmed
Creatinine	T2	$\pm 15\%$	Confirmed
Cystatin C	T0, T1	$\pm 20\%$	Confirmed
Urea	T2	$\pm 20\%$	Confirmed
Nitrogen balance ($\Delta T2-T0$)	—	$\pm 20\%$	Confirmed
Body cell mass (BCM)	T1, T2	$\pm 10 \text{ CU}$	Confirmed
$\text{Na}^+, \text{K}^+, \text{Ca}^{2+}, \text{Cl}^-$	T2	$\pm 3 / \pm 0.3 / \pm 0.1 / \pm 3$	Confirmed
Malondialdehyde (MDA)	T0, T1, T2	$\pm 20\%$	Not confirmed

Note. Orn/Cit, Arg/Orn, Arg/Cit — ornithine/citrulline, arginine/ornithine, arginine/citrulline. GMR — geometric mean ratio. * — Specific Δ values for each measure were set prior to the study and are detailed in the «Statistical Analysis» section. All p values for TOST were reported according to the statistician's report; the TOST analysis was conducted on a combined sample of 120 patients (PP analysis — for the corresponding measure). For BCM, the report used a broad clinically acceptable Δ limit of 10 arbitrary/conventional units, reflecting inter-device and inter-patient variability.

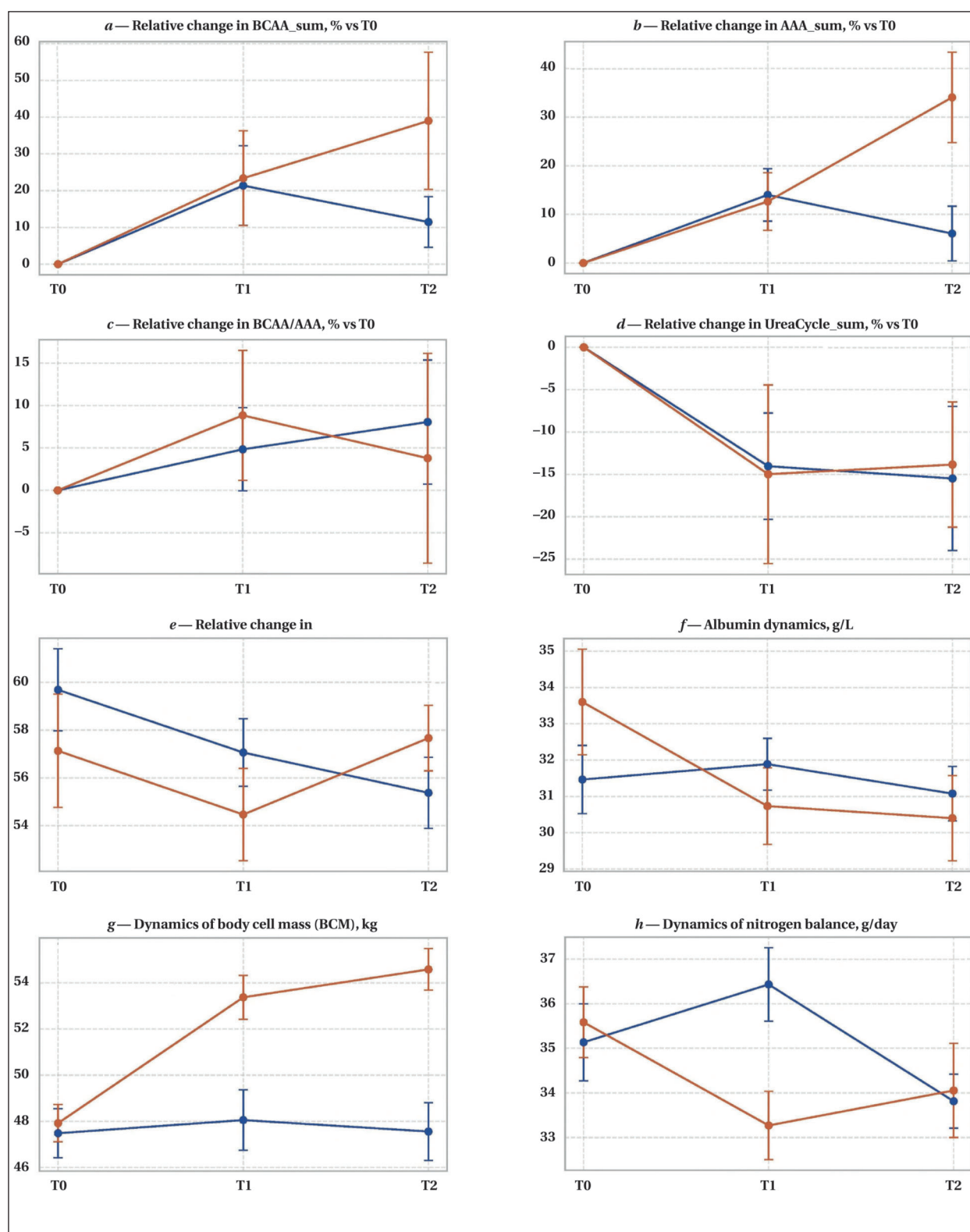
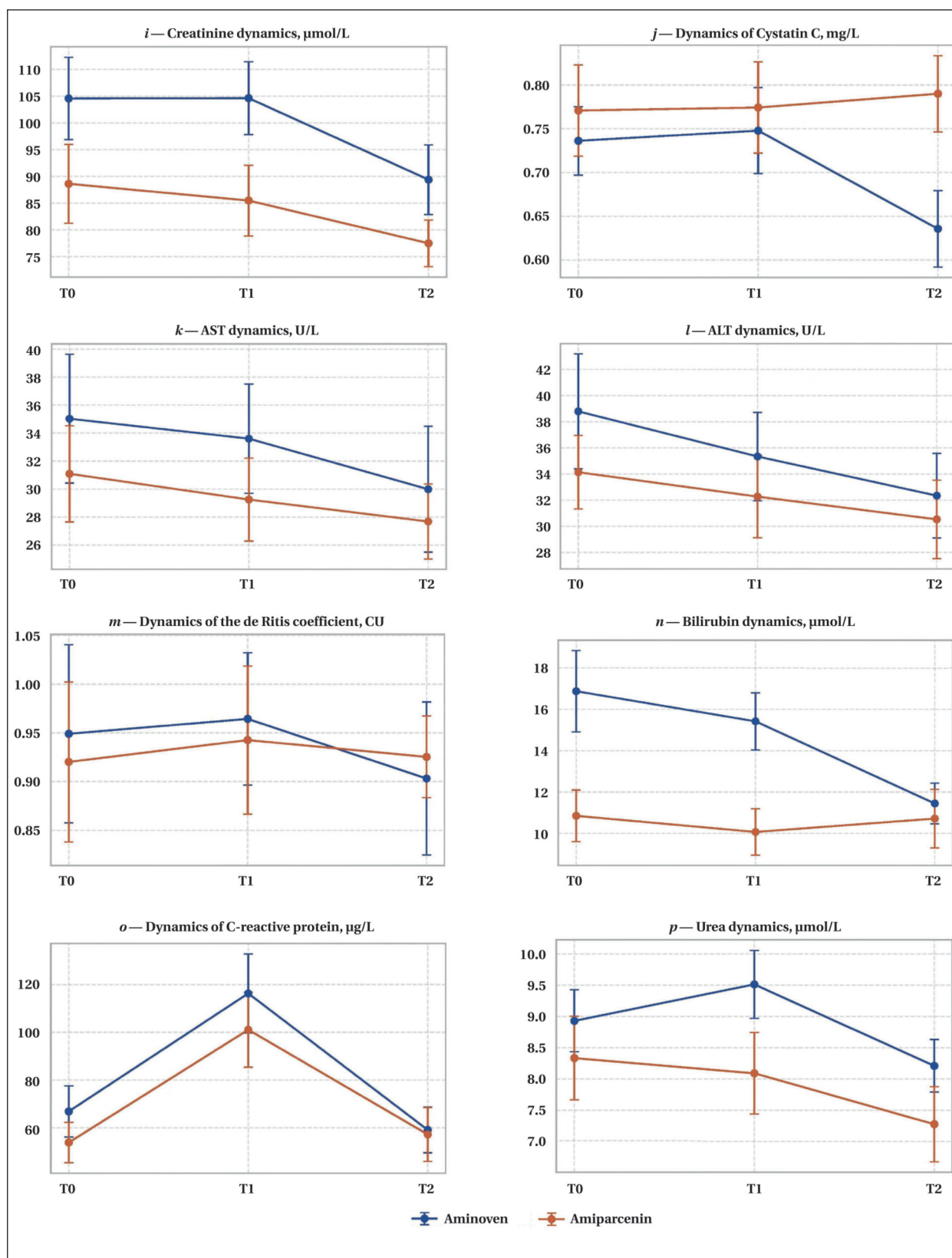


Fig. 3. Dynamics of indicators in the Aminoven and Ampiparcenin groups.

Note. The X-axis represents the reference time points of the study. Indicators: amino acid profile — *a-d*; nutritional status and body composition — *g*; kidney function — *i, j*; liver function — *k-n*; other laboratory and clinical markers — *e, f, h, o, p*.

ratio (Fisher's coefficient) remained within the range typical for patients without severe liver failure at all reference time points. According to Friedman's criterion, no significant changes in the coefficient were observed in any group ($p > 0.3$);

no intergroup differences were found either ($p > 0.2$ at each reference time point). Repeated measures ANOVA did not show a significant effect of the «group», «period», and «group × period» factors on Fisher's coefficient.



Continuation of Fig. 3.

The TOST analysis demonstrated that the BCAA/AAA ratio at all reference time points met the equivalence criteria for two regimens: 90% CI of the difference between groups completely fell within the pre-defined interval $[-\Delta; +\Delta]$.

Urea cycle indicators (UreaCycle_sum, Fig. 3, d).

The composite of UreaCycle_sum changed moderately in both groups, without a clear unidirectional trend according to Friedman's criterion. However, differences in absolute values of UreaCycle_sum

were observed between the groups, and the TOST analysis did not confirm equivalence for this composite measure.

The ratios of ornithine/citrulline, arginine/ornithine, and arginine/citrulline:

- did not change significantly according to Friedman's criterion;
- did not differ between groups at reference time points;
- met equivalence criteria according to TOST in all reference time points.

Considering comparable levels of creatinine and nitrogen balance, the differences in UreaCycle_sum were regarded as having no clinically significant impact on nitrogen utilization.

Nutritional indicators and body composition.

Total protein and albumin (Fig. 3, e, f). By the fifth postoperative day, the concentration of total protein and albumin significantly changed compared to baseline values in both groups.

In the Aminoven group, total protein and albumin increased across all pairs of reference time points (T0–T1, T1–T2, T0–T2), with $p < 0.001$ for most comparisons. In the Amiparcenin group, the dynamics were comparable, with statistically significant increases between all pairs of reference time points.

No intergroup differences in absolute values of total protein and albumin were found at each reference time point. The TOST analysis indicated that the differences between the regimens for these indicators were within the pre-defined range of clinically acceptable differences, and equivalence was confirmed (Table 3).

Body cell mass according to bioimpedance analysis (Fig. 3, g). In the Amiparcenin group, bioimpedance analysis showed a clear positive trend in body cell mass:

- Friedman criterion: $p = 0.00001$;
- Pairwise comparisons (Wilcoxon) confirmed the increase in BCM between T0 and T1, T1 and T2, and between T0 and T2 ($p < 0.01$).

In the Aminoven group, the overall dynamics of BCM according to the Friedman criterion were less pronounced and did not reach statistical significance ($p = 0.18435$), although there was a trend toward its increase.

When comparing between groups, the baseline BCM values were comparable ($p = 0.770$), but on days 2 and 5, the values in the Amiparcenin group became significantly higher (T1: $p = 0.004816$; T2: $p = 0.000139$). Additionally, TOST analysis with a wide Δ showed formal equivalence in BCM between the groups, indicating that even the more significant BCM increase in the Amiparcenin group remained within the pre-defined range of clinically acceptable differences.

Nitrogen balance (Fig. 3, h). The nitrogen balance in the Amiparcenin group significantly im-

proved over five days (Friedman criterion $p = 0.00001$), while the Aminoven group showed a statistically insignificant trend ($p = 0.20584$). In intergroup comparisons:

- At T0, no differences in nitrogen balance were observed ($p = 0.6726$);
- At T1 and T2, significant differences were found ($p = 0.0392$ and $p = 0.001556$, respectively), with more favorable values in the Amiparcenin group. TOST analysis indicated that the difference in changes $\Delta(T2-T0)$ between the groups remained within the predefined clinical range, and the equivalence in nitrogen balance was generally maintained (Table 3).

Kidney function. The concentrations of creatinine and cystatin C in both groups remained within reference values and did not show a trend toward significant increase by the fifth day of follow up (Figure 3, i, j). No intergroup differences were noted for these parameters. The TOST analysis confirmed the equivalence of the effects of both regimens on kidney function markers at each reference time point (Table 3).

Liver function and electrolytes. Liver function markers (AST, ALT, De Ritis ratio, bilirubin concentration, Figure 3, k, l, m, n) moderately changed in the early postoperative period, but overall remained within acceptable limits and did not differ between groups. The TOST analysis showed that the values of these markers in both regimens at all reference time points were within clinically acceptable ranges, indicating no significant hepatotoxic effect from either solution.

Equivalence was also statistically confirmed for sodium, potassium, calcium, and chloride concentrations at all reference time points within predefined clinically relevant limits Δ (TOST, $p < 0.01$; Table 3).

Discussion

The main practical question in the context of this research is not so much identifying the fundamental differences between two specific formulations, but rather assessing whether different standard amino acid solutions (with variations in amino acid profiles and the presence or absence of sorbitol) can be regarded as comparable in terms of clinical and biochemical effects components of combined nutritional support for patients with abdominal cancers.

It's important to emphasize that the study predominantly included stable patients with excess body weight (average BMI of 25.3–27.6 kg/m²) and no significant biochemical abnormalities; therefore, the results obtained primarily apply to this category of patients. In a limited number of studies comparing different amino acid formulations (in patients with severe neurosurgical conditions, in post-bone mar-

row transplant patients, and in premature infants), the amino acid profile, nitrogen balance, and specific clinical indicators were analyzed [12, 14–19]. Most contemporary research on parenteral nutrition focuses on comparing routes and regimens (enteral vs. parenteral, premixed vs. compounded mixtures) or on specialized formulations (BCAAs, glutamine, omega-3 enriched formulas) [5, 12, 13, 20, 21]. There are virtually no studies originally designed as equivalence trials of standard amino acid solutions using the TOST approach and predefined clinical Δ s.

The results obtained are generally consistent with the current recommendations from ESPEN, ESMO, the Ministry of Health of the Russian Federation, and the Federation of Anesthesiologists and Reanimatologists [1–3, 5–11], which emphasize that for cancer patients without severe liver failure, the key factors for outcomes are:

- Timely initiation of nutrition (preferably within the first 24 hours after surgery);
- Achieving the target protein and energy intake of nutrients;
- Prioritizing the enteral route with the addition of a parenteral component when it is not possible to meet needs solely through the gastrointestinal tract.

In our study, the amino acid profile and the Fisher ratio (the BCAA/AAA ratio) remained within clinically acceptable ranges and did not differ between groups. This aligns with the concept that, in the absence of liver function decompensation, standard amino acid solutions typically provide an adequate BCAA/AAA ratio, and specialized BCAA-enriched mixtures are primarily warranted for patients with severe liver failure [5, 10–13]. It is important to emphasize that the Fisher ratio was not used as a surrogate for hepatic encephalopathy but rather as an integral marker of amino acid balance and metabolic stress.

Differences in the absolute values of BCAA_sum and AAA_sum between solutions reflect the characteristics of their composition and the baseline amino acid levels, which is expected based on comparative studies [12, 14, 16–19], but did not lead to development of a clinically significant amino acid imbalance. This is supported by the equivalence of the BCAA/AAA ratio and the index indicators of the urea cycle (ornithine/citrulline, arginine/ornithine, arginine/citrulline), as well as comparable values for nitrogen balance and markers of liver and kidney function.

The composite indicator of UreaCycle_sum differed statistically between groups and did not meet the formal criteria for equivalence according to the TOST analysis. However, the index ratios of urea cycle metabolites remained stable and equivalent, while levels of creatinine, urea, and nitrogen balance were comparable. Therefore, this allows to

conclude that the observed differences in UreaCycle_sum have no clinically significant impact on nitrogen utilization.

The use of both regimens was accompanied by expected changes in total protein and albumin, characteristic of the early postoperative period and the nutritional support provided, as well as by improvement in nitrogen balance. These data are consistent with the results of randomized studies and meta-analyses demonstrating that adequate perioperative nutritional support (enteral, parenteral, or combined) reduces the rate of complications and length of hospital stay in patients with gastrointestinal neoplasms [4, 5, 11, 21, 22]. Furthermore, the results clarify that within the same combined support protocol, the choice between two standard amino acid solutions differing in compositions and presence of sorbitol does not have a significant impact on clinical and biochemical parameters in patients without severe liver and kidney failure.

A more pronounced increase in body cell mass and a significant improvement in nitrogen balance in Amiparcenin (containing sorbitol) group may indicate a somewhat faster gain in functionally active lean mass and a reduction of catabolism rates in the early postoperative period. Considering that reduced body cell mass and unfavorable bioimpedance indicators are associated with an increased risk of complications and worse survival in patients undergoing cancer surgery [4, 24], this observation appears clinically promising. However, from the perspective of formal TOST analysis, even these differences remain within the pre-defined limits Δ for body cell mass, allowing these regimens to be interpreted as clinically comparable regarding this indicator. More extensive and longer studies are needed to assess the impact on «solid/robust» outcomes (Clavien-Dindo complications, survival, quality of life).

The conducted study adds to the limited literature comparing various amino acid compositions and parenteral regimens, often in specialized populations (premature infants, severe neurosurgical patients, patients after bone marrow transplantation) [12, 14, 16–19]. Unlike the referenced studies, this study evaluated the comparability of clinical and biochemical effects of two standard amino acid solutions in a group of adult patients undergoing surgery for abdominal neoplasms, using strictly standardized combined nutritional support protocol and a formal TOST approach.

The limitations of the study include:

- A focus on intermediate biochemical and nutritional indicators over a short follow up period (the first five days post-surgery);
- Lack of direct assessment of long term clinical outcomes (mortality, severe complications according to Clavien-Dindo classification, survival, quality of life);

- Exclusion of certain patients from the per-protocol analysis after randomization due to development of severe complications in the early postoperative period, which may lead to systematic error (attrition bias) and skew the results towards a more favorable outcome, despite conducting an ITT- analysis;

- Absence of prospective registration of the protocol in publicly accessible clinical trial registries;

- Selection of a relatively homogeneous population (stage I–III of the neoplastic progression, without severe comorbid liver and kidney pathology), which limits the extrapolation of results to more severely affected patient categories.

These limitations must be taken into account when interpreting the results and planning further research.

In the absence of specific restrictions, the choice of a particular amino acid mixture will most likely be determined by availability, cost, and logistical factors. In special clinical situations (for example, in cases of severe liver failure, severe cachexia, or multiple comorbidities), it is advisable to tailor the approach and continue the accumulation of data.

Conclusion

In patients undergoing surgery for abdominal neoplasms, standardized combined nutritional support during the early postoperative period including amino acid solutions Amiparcenin and Aminoven 10% is associated with comparable amino acid indices (including the BCAA/AAA ratio) and nitrogen metabolism indicators.

Differences in absolute total concentrations of individual amino acids (BCAA_sum, AAA_sum) and composite metabolites of the urea cycle (Urea-Cycle_sum) do not lead to clinically significant amino acid imbalances or significant changes in liver and kidney function in the early postoperative period.

Both solutions can be considered as interchangeable components of the parenteral part of combined nutritional support for patients undergoing surgery for abdominal neoplasms in the early postoperative period. However, any final conclusion regarding complete clinical equivalence should be approached with caution following lack of control over the use of fat emulsions in this study, which could introduce additional variability in energy and metabolic balance.

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The Effect of FTY720 on Brain Lipids, Ceramide, and TNF- α in Acute Cerebral Ischemia in Rats

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Summary

Modulation of sphingosine-1-phosphate receptors exerts various neuroprotective effects under conditions of cerebral ischemia. In this study, we investigated the relationship between the lipid composition of brain tissue, lipid signaling, and the content of the proinflammatory cytokine TNF α , as well as the expression of its receptor, TNFR1.

Objective. In a rat model of acute cerebral ischemia, to evaluate the effects of FTY720 on the lipid composition of brain tissue, ceramide concentration, and the expression of key enzymes involved in its synthesis, as well as on the contents of TNF- α and its receptor TNFR1.

Materials and Methods. The study was conducted on 37 male white non-linear rats weighing 180–230 g. Acute cerebral ischemia was induced by a combined procedure involving irreversible ligation of the left common carotid artery and reversible ligation of the right common carotid artery. The animals were divided into three groups: sham-operated, rats with acute cerebral ischemia, and rats with ischemia following prior administration of FTY720 (fingolimod). On the third day of observation, neurological deficits in surviving animals were assessed using the Garcia scale. The sphingolipid and phospholipid composition of brain tissue was examined using thin-layer chromatography. Ceramide concentration, the expression of enzymes involved in its biosynthetic, TNF α , and TNFR1 concentrations were evaluated using immunofluorescent microscopy.

Results. Pretreatment with fingolimod was associated with better survival rates: 31% in the FTY720 group vs. 20% in the Ischemia group ($p=0.043$). Functional impairments on the Garcia scale were significantly less severe in the FTY720 group than in the Ischemia group: 14 [13.5; 15] vs. 11 [10; 12.5] scores ($Me [Q1; Q3]$), $p<0.01$). FTY720 group also demonstrated a decrease in ceramide concentration in the brain tissue compared to the Ischemia group ($p=0.0005$), along with downregulated expression of aSMase ($p=0.0012$), nSMase ($p=0.0003$) enzymes involved in its synthesis, of SPT ($p=0.0002$), and CerS ($p=0.0001$), a decrease in pro-inflammatory cytokine TNF α ($p=0.0003$) concentration and normalized expression of its receptor TNFR1.

Conclusion. Preservation of phospholipid composition and reduction in the excessive production of ceramide and pro-inflammatory cytokines in the brain tissue are associated with less severe neurological deficits and improved survival rates in rats during the acute phase of cerebral ischemia.

Keywords: FTY720; ceramide; TNF α ; TNFR1; cerebral ischemia; phospholipids; sphingolipid signaling

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Neuronal inflammation in acute cerebral ischemia develops within minutes of the onset of injury and is characterized by activation of resident immune cells (astrocytes and microglia), infiltration of brain tissue by peripheral immune cells, release of pro-inflammatory cytokines, chemokines, and reactive oxygen species, and by migration of effector T cells [1].

There is evidence regarding the role of the sphingosine-1-phosphate (S1P) pathway in neuroprotection during ischemia. S1P synthesis involves sphingosine kinases SphK1 and SphK2, and the non-selective S1P receptor modulator fingolimod (FTY720) exhibits neuroprotective activity by influ-

encing neuronal inflammation [2]. The S1P receptor family includes five subtypes (S1P1–S1P5) with different effects. S1P1 is the most highly expressed; its activation increases intracellular calcium concentration, triggers anti-apoptotic mechanisms, cytoskeletal reorganization, and cell migration [3]. FTY720 has high affinity for S1P1, and studies in rats show that its use reduces infarct volume, the severity of neurological deficits, and neuronal apoptosis [4]. However, some data suggest that the neuroprotective properties of FTY720 may be associated with the inhibition of S1P1 [5].

The pathophysiological role of S1P1 in ischemia is primarily attributed to its modulation of neuronal inflammation: it reduces the production of pro-in-

flammatory cytokines, microglial activity, and the expression of MAPK and NF- κ B, while decreasing blood-brain barrier (BBB) permeability [6]. Inhibition of S1P2 also reduces infarct volume, cerebral edema, and BBB permeability, likely through its effects on matrix metalloproteinase-9 and the migration of neuronal progenitors [7]. S1P3 inhibition is accompanied by reduction in cellular infiltration and production of pro-inflammatory mediators, by synthesis of anti-inflammatory cytokines, and a decrease in microglial activation [8]. The role of S1P4 and S1P5 has been studied to a limited extent, although it is known that activation of S1P4 stimulates neutrophil migration, and inhibition of S1P5 may increase levels of pro-inflammatory cytokines [9].

Ceramide is a known biochemical precursor of S1P. Pro-inflammatory mediators, hypoxia, and oxidative stress stimulate synthesis of ceramide, which acts as a pro-apoptotic signal and regulates autophagy, the inflammatory response, and cell differentiation [10].

Three main mechanisms of FTY720-mediated neuroprotection have been described: interaction with S1P receptors on lymphocytes, leading to lymphopenia and reduced lymphocytic infiltration; interaction with S1P receptors on neurons, microglial cells, astrocytes, oligodendrocytes, and endothelial cells; and a receptor-independent mechanism in which FTY720 binds to intracellular proteins, modulating signaling pathways and epigenetic transcription [11].

Objective of the study: to evaluate the effect of FTY720 on the survival and neurological deficits in rats, and assess the phospholipid and sphingolipid composition of brain tissue, concentration of ceramide and expression of enzymes involved in its synthesis in the catabolic and de novo pathways, as well as levels of TNF α and its receptor TNFR1 in a model of acute cerebral ischemia.

Materials and Methods

The study was conducted on 37 male white nonlinear rats weighing 180–230 g. All procedures involving the animals were approved by the local ethics committee of Izhevsk State Medical University (Protocol No. 736/1 dated May 11, 2022). The study was conducted in accordance with international standards (Recommendation No. 33 of the Board of the Eurasian Economic Commission dated November 14, 2023, «On the Guidelines for the Care and Use of Laboratory (Experimental) Animals in Preclinical (Nonclinical) Research»), the ARRIVE 2.0 guidelines, the Guide for the Care and Use of Laboratory Animals (NRC, 2011), and Directive 2010/63/EU, in compliance with the 3R principles. Adequate anesthesia was used, and humane endpoints were determined.

Modeling of acute cerebral ischemia. The animals were fasted the day before surgery. The pro-

cedure was performed under strict aseptic conditions. Anesthesia was induced with a combination of zolazepam and tiletamine (Zoletil 100, Virbac, France) at a dose of 30 mg/kg body weight. The surgical site was shaved and treated with a 5% alcohol solution of iodine. A midline incision was made on the ventral surface of the neck, after which the carotid arteries were dissected layer by layer from the surrounding soft tissues and neural structures. Loose ligatures were applied to both carotid arteries. The left carotid artery was ligated, and a bulldog clamp was applied to the right carotid artery for 30 minutes. Upon completion of the procedure, the wound was sutured layer by layer after being treated with a 0.05% aqueous solution of chlorhexidine digluconate [12].

The animals were randomly assigned to three groups. The first group (Ischemia) consisted of rats in which acute cerebral ischemia was induced. Of the 85 animals that underwent surgery in this group, 17 survived, corresponding to a 20% survival rate. The second group (FTY720) consisted of animals that were administered fingolimod (FTY720) at a dose of 1 mg/kg body weight prior to ischemia induction. Of the 35 animals in the second group, 11 survived (31% survival rate). The third group consisted of sham-operated animals (SO) that underwent all stages of the surgical procedure without carotid artery ligation ($n=9$).

Fingolimod was administered at a dose of 1 mg/kg body weight immediately prior to the induction of acute ischemia. The choice of dose and timing of administration was based on data from systematic reviews demonstrating a reduction in the volume of ischemic lesion, the severity of cerebral edema, and the degree of behavioral deficits when using this dosing regimen [13].

On day 3, survival and neurological status were assessed using the Garcia scale [14], after which the animals were euthanized under general anesthesia.

Macroscopic visualization of the ischemic lesion. The animal was anesthetized with a combination of zolazepam and tiletamine. Once the desired depth of anesthesia was achieved, access to the heart was established, and a cannula was inserted into the left ventricular cavity. Systemic perfusion was performed with a 4% solution of 2,3,5-triphenyltetrazolium chloride prepared in phosphate-buffered saline (PBS; composition, mM: 3.2 NaH₂PO₄, 0.5 K₂HPO₄, 1.3 KCl, 135 NaCl; pH 7.4). After decapitation, the brain was removed and fixed in a 10% formalin solution, and after 24 hours, 1-mm-thick coronal sections were prepared.

Identification of the phospholipid and sphingolipid composition of brain tissue. Brain tissue was homogenized, after which the homogenate was incubated in a 2:1 chloroform/methanol mixture using the Folch method to ensure complete lipid extraction. Lipid fractions were separated by thin-layer chromatography in chambers containing the

appropriate solvent systems. A chloroform/methanol/glacial acetic acid/distilled water system (60:50:1:4) was used to isolate phospholipid fractions, and a butanol/glacial acetic acid/distilled water system (3:1:1) was used to separate sphingolipids. Samples were applied to Merck UV-labeled chromatographic plates (TLC Silica gel 60 F254, 20×20 cm). Lipids were visualized in iodine vapor, and quantitative analysis was performed using a «Sorbfil» densitometer [16].

Immunofluorescence microscopy study. Under general anesthesia with zolazepam/tiletamine, transcardial systemic perfusion was performed using phosphate-buffered saline, followed by a 4% paraformaldehyde fixative solution prepared on a PBS base. The brain was removed, placed in 4% paraformaldehyde for 2 hours for additional fixation, then kept in a 30% sucrose solution for 24 hours and frozen using dry ice.

In the next step, 14-micrometer-thick coronal sections of the brain were prepared using an HM525 NX Cryostat (Thermo Fisher Scientific, USA). The resulting sections were mounted on adhesive-coated slides (Thermo Fisher Scientific, Waltham, MA, USA).

The sections were incubated in a permeabilization solution (1% Triton X-100 in PBS containing 5% bovine serum albumin) for 20 minutes. They were then incubated in a solution of primary antibodies: anti-TNFR1, anti-TNF α , anti-aSMase, anti-nSMase (monoclonal rabbit IgG, 1:200 dilution, Affinity Biosciences), and anti-Cer (monoclonal mouse IgG, 1:200, ALX-804-196-T050, Enzo Life Sciences, USA), anti-SPT2, and anti-CerS1 (rabbit IgG, 1:200, Abcam) in the presence of 5% bovine serum albumin. Incubation was performed overnight. After washing in PBS, the sections were incubated in secondary antibody solutions: anti-rabbit Alexa Fluor 488 (goat IgG, 1:300, Abcam) and anti-mouse Alexa Fluor 647 (goat IgG, 1:300, Abcam) for 1 hour at room temperature under light-protected conditions [17].

Immunofluorescence in brain sections was analyzed using the DS-Fi3 EF-2E attachment for the Nikon Eclipse E200 microscope, NIS-Elements D software (Nikon Instruments Inc., USA), and ImageJ. The intensity of expression was calculated based on the fluorescence intensity over a standard area of 0.1 mm².

Statistical data were analyzed using IBM SPSS Statistics 23 and reported as the median and interquartile range (*Me* [*Q1*; *Q3*]). The normality of the distribution was assessed using the Shapiro–Wilk test. Due to deviation of indicators distribution from the normal, nonparametric tests were used for intergroup comparisons. For intergroup comparisons of three independent groups, the Kruskal–Wallis *H*-test was used. When statistically significant differences were identified, post-hoc analysis was performed via pairwise comparisons of groups using the Mann–Whitney *U*-test with Bonferroni correction for multiple comparisons. Fisher's exact test was used to analyze differences in frequency characteristics (proportions of surviving animals) between the groups. Differences were considered statistically significant at a *p*-level < 0.05. The results were presented graphically as box-and-whisker plots with statistical significance levels indicated.

Results

Animals that received FTY720 prior to the induction of acute cerebral ischemia showed a statistically significantly higher survival rate compared to animals in the Ischemia group: 31% and 20%, respectively, *p* = 0.043.

Pretreatment with FTY720 also contributed to a reduction in neurological deficits. Functional impairments on the Garcia scale were significantly less obvious in the FTY720 group with the median of 14 scores (*Me* [*Q1*; *Q3*] = 14 [13.5; 15]) vs. 11 scores (*Me* [*Q1*; *Q3*] = 11 [10; 12.5]) in the Ischemia group. A comparison of the three groups using the Kruskal–Wallis test revealed statistically significant

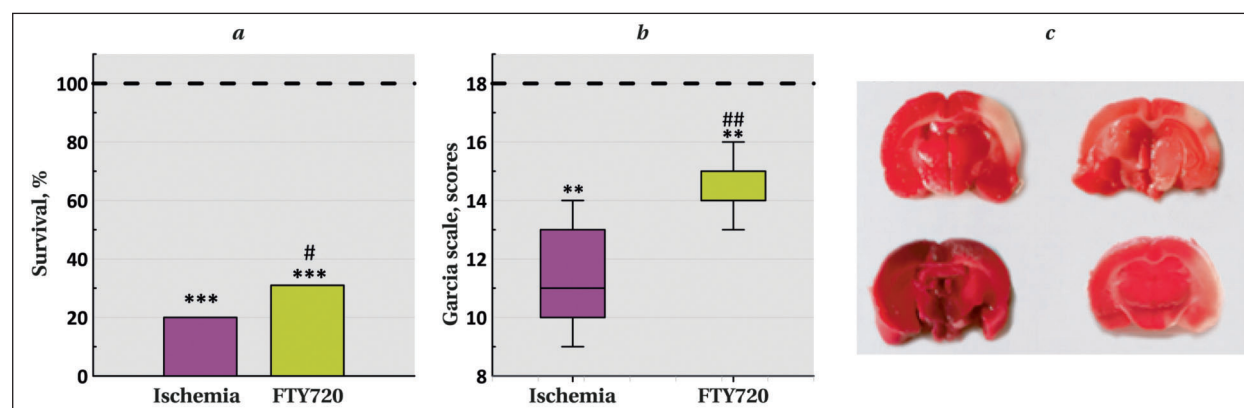


Fig. 1. Survival (a), functional deficit (b), and visualization of the ischemic lesion using 2,3,5-triphenyltetrazolium chloride staining (c) in a model of acute cerebral ischemia and ischemia after pretreatment with FTY720.

Note. The dotted line represents the SO group. ** — *p* < 0.01; *** — *p* < 0.001 — compared to SO group animals; # — *p* < 0.05; ## — *p* < 0.01 — compared to the Ischemia group animals.

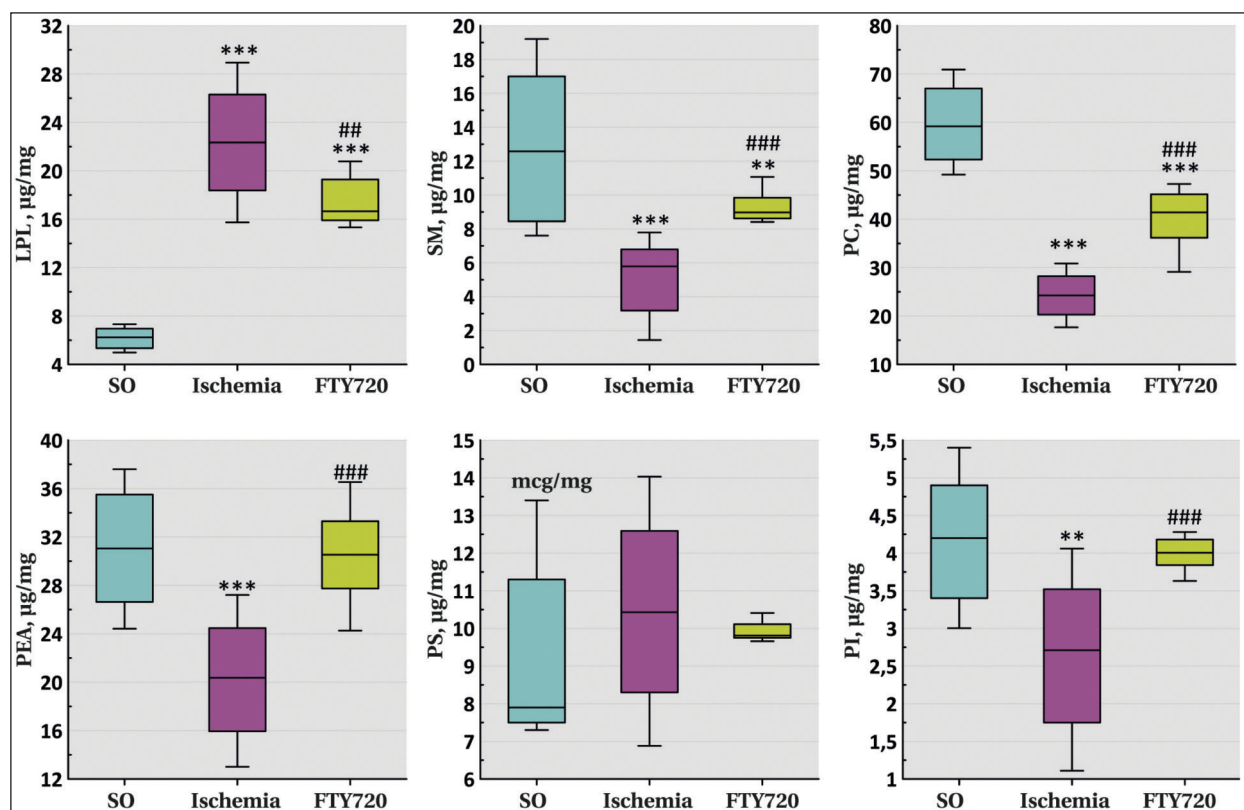


Fig. 2. Concentrations of major phospholipid fractions in the study groups.

Note: LPL — lysophospholipids; SM — sphingomyelin; PC — phosphatidylcholine; PEA — phosphatidylethanolamine; PS — phosphatidylserine; PI — phosphatidylinositol. * — $p < 0.01$; *** — $p < 0.001$ — compared to SO animals; # — $p < 0.01$; ### — $p < 0.001$ — compared to the group of animals that did not receive FTY720.

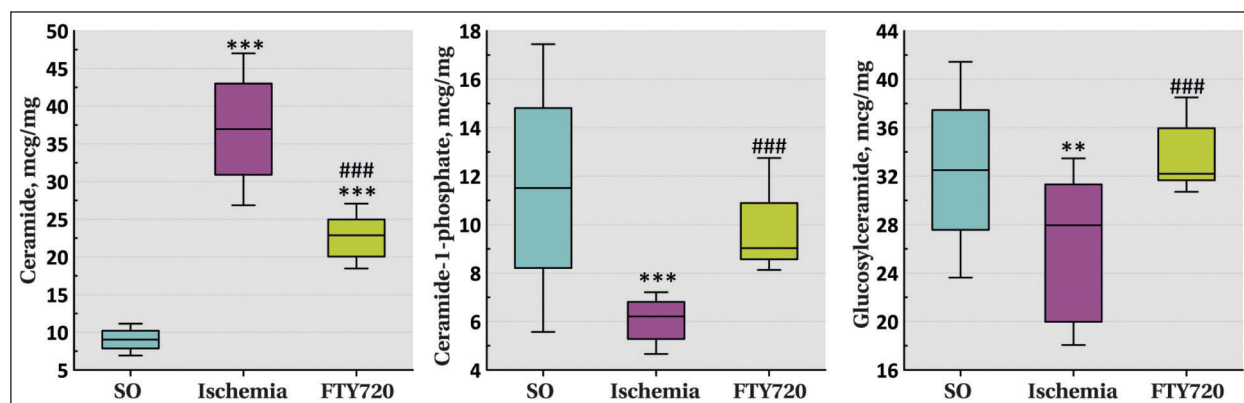


Fig. 3. Concentrations of ceramide and its metabolites in the study groups.

Note. * — $p < 0.01$; *** — $p < 0.001$ — compared with SO group; ### — $p < 0.001$ — compared with Ischemia group.

differences ($p = 0.000008$). The subsequent pairwise comparison of the groups using the Mann–Whitney test with Bonferroni correction revealed higher Garcia scale scores in the FTY720 group compared to the Ischemia group ($p = 0.0019$). No neurological deficits were found in the SO group.

Fig. 1, *a* shows survival rates, while Fig. 1, *b* presents data on neurological deficits according to the Garcia scale. Fig. 1, *c* shows coronal sections of the forebrain of rats stained with 2,3,5-triphenyltetrazolium chloride (TTC). The bottom row on the

left shows a brain section from a sham-operated (SO) animal: the tissue is uniformly stained red, indicating its viability and preservation of dehydrogenase activity. The remaining sections (top row and bottom row on the right) show white, unstained areas corresponding to the zone of ischemic damage, where enzymatic activity has been lost.

Fig. 2 shows changes in the phospholipid composition of brain tissue during acute cerebral ischemia. Three-day ischemia resulted in a significant decrease in concentrations of the main phospholipid

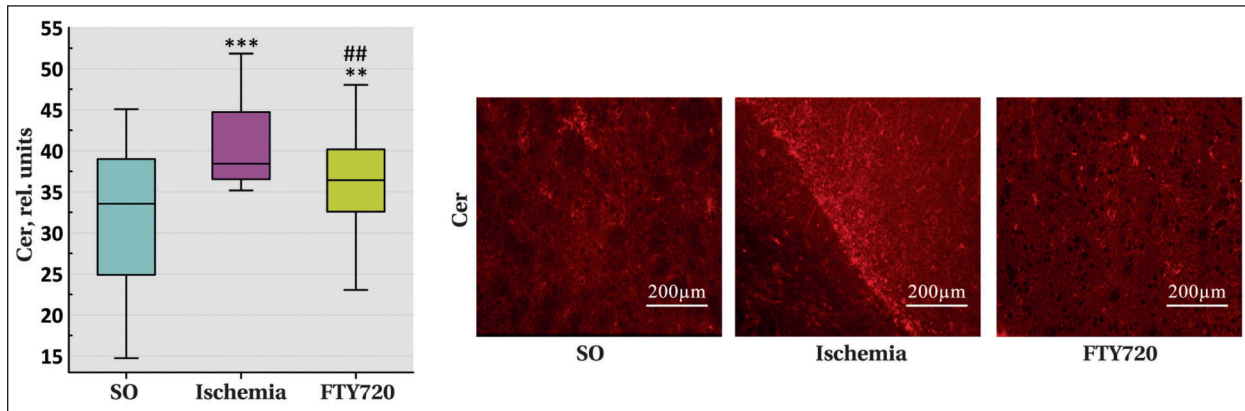


Fig. 4. Ceramide expression in the study groups.

Note. ** — $p < 0,01$; *** — $p < 0,001$ — по сравнению с ЛО животными; ## — $p < 0,01$ — по сравнению с группой животных с ишемией.

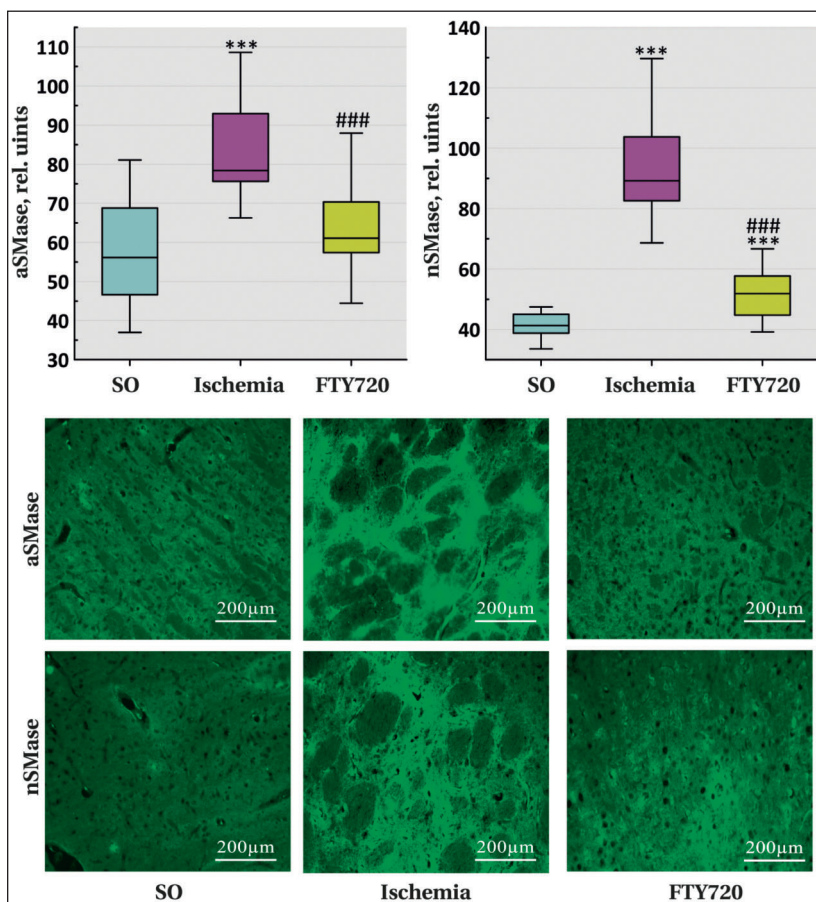


Fig. 5. Expression of aSMase and nSMase in the studied groups.

Note. *** — $p < 0.001$ — compared to SO animals; ### — $p < 0.001$ — compared to the group of animals with ischemia.

fractions: phosphatidylethanolamine (PEA) by 34.9% ($p = 0.0002$), phosphatidylcholine (PC) — by 59.3% ($p = 0.000012$). Concentration of sphingomyelin (SM) dropped by 60.2% ($p = 0.0003$), and of phosphatidylinositol (PI) — by 37% ($p = 0.00012$). There was a concomitant accumulation of lysophospholipids (LPL) with 3.79-fold increase of LPL concentration ($p = 0.0002$).

In animals pretreated with FTY720, SM concentration was 1.9-fold higher ($p = 0.0004$), and concentration of PC — 1.7-fold higher ($p = 0.0001$) compared to the Ischemia group; however, these values did not reach the levels observed in the SO group. PEA and PI concentrations in the FTY720 group increased 1.5-fold ($p = 0.0002$ and $p = 0.001$, respectively) and were comparable to those in the SO group.

In the Ischemia group ceramide concentration in brain tissue increased 4.2-fold ($p = 0.0003$) compared to the SO group, while ceramide-1-phosphate concentration decreased by 47.4% ($p = 0.0008$), and that of glucosylceramide decreased by 14.1% ($p = 0.009$).

Pretreatment with FTY720 mitigated these effects, and ceramide concentration was 1.6 times lower in the FTY720 group vs the Ischemia group ($p = 0.0005$), while concentrations of ceramide-1-phosphate and glucosylceramide were comparable to those in the SO group. Fig. 3 presents obtained data on concentrations of ceramide and its metabolites.

Immunofluorescence microscopy findings confirmed that pretreatment with FTY720 downregulated ceramide synthesis in the ischemic brain.

Ceramide concentration was 1.3 times higher in Ischemia group compared to the SO group ($p = 0.0006$), while in rats pretreated with FTY720, it was 1.3 times lower than in the Ischemia group ($p = 0.002$), although remained significantly higher

than in the SO group animals. Data on ceramide content in brain tissue of all studied groups is presented in Fig. 3, 4.

In the sphingomyelinase pathway, ceramide is formed by hydrolysis reaction, catalyzed by acidic and neutral sphingomyelinases aSMase and nSMase. In acute cerebral ischemia, the expression of aSMase and nSMase significantly increased: by 1.4 times ($p=0.0001$) and by 1.8 times, respectively ($p=0.0004$). Pretreatment with FTY720 resulted in a decrease of aSMase expression to a level comparable with SO group, and 1,2-fold decrease in nSMase, although, not reaching the values in the SO group. Sphingomyelinases aSMase and nSMase expression in all studied groups is shown in Fig. 5.

Serine palmitoyltransferase (SPT) and ceramide synthase (CerS) enzymes expression in acute cerebral ischemia was evaluated to characterize «de novo» synthesis of ceramide. SPT expression in the Ischemia group was increased by 1.5 times ($p=0.0001$), and CerS expression — by 1.9 times ($p=0.0001$) compared to the SO group. Pretreatment with FTY720 prevented an increase in the expression of both enzymes, bringing it to values comparable to those in the SO animals. The results are presented in Fig. 6.

Evaluation of pro-inflammatory TNF α cytokine and its receptor TNFR1 revealed 1.6-fold increase in TNF α concentration ($p=0.0001$), and 1.9-fold increased expression of TNFR1 ($p=0.0001$) in the Ischemia group compared to the SO group. Pretreatment with FTY720 led to a 1.3-fold decrease in TNF α production compared to the Ischemia group ($p=0.0003$), although its concentration was higher than in controls. TNFR1 density in the FTY720 group also decreased to the levels close to the SO group. The effect of FTY720 on TNF α and TNFR1 expression is shown in Fig. 7.

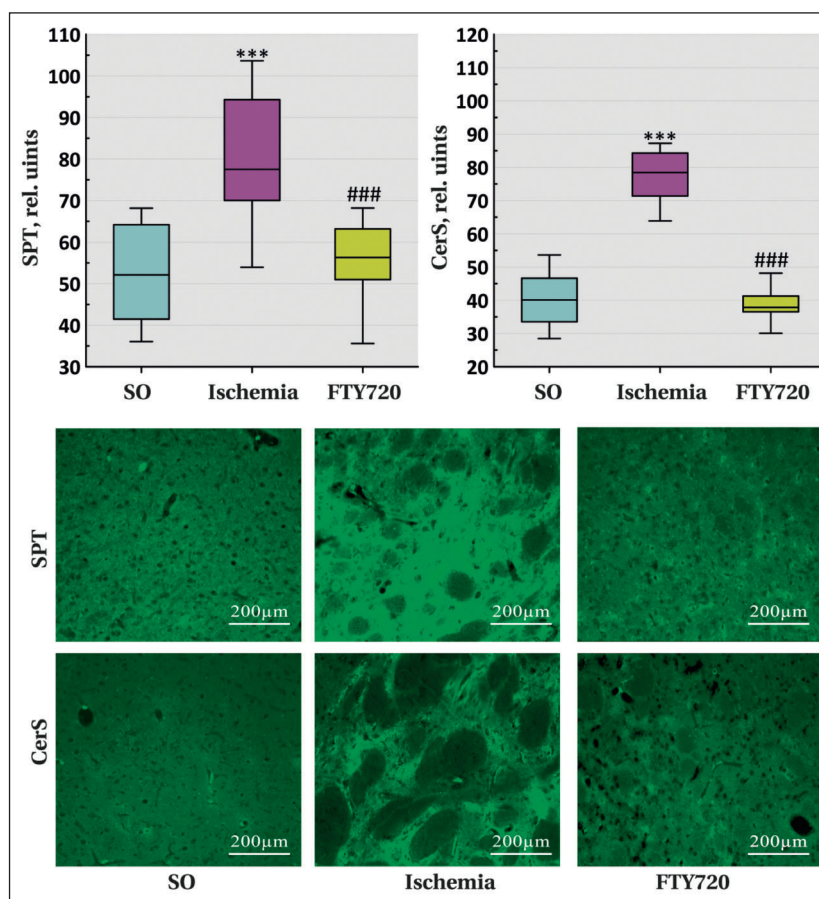


Fig. 6. Expression of SPT and CerS in the studied groups.

Note. *** — $p<0.001$ — compared to SO animals; ### — $p<0.001$ — compared to the group of animals with ischemia.

species and pro-inflammatory cytokines, primarily IL-1, TNF α , and IFN γ [18].

Further in this continuum oxylipins and lysophospholipids with potent biological signaling functions are generated following activation of cyclooxygenase, lipoxygenase, and cytochrome-P450 monooxygenase. Established effects of lysophosphatidic acid and sphingosine-1-phosphate include stimulation of neuronal inflammation and leukocyte infiltration. Production of these compounds is downregulated by FTY720, mainly due to a decrease in the inflammatory response, a reduction in T-cell and neutrophil infiltration, and a decrease in microglia activation [19].

The experiment of G. Payne et al. (2007) on a mast cells culture demonstrated FTY720 potential to inhibit phospholipase A₂ activity, thereby reducing secretion of prostaglandins and thromboxanes, which led to a decrease in inflammation and hydrolysis of cell membranes [20].

In our experiments a decrease in SPT expression was documented. Published data does not support a direct inhibitory effect of FTY720 on this enzyme, contrary to its natural precursor, myriocin, which is a known SPT inhibitor [21]. The myocardial is-

Discussion

Membrane lipids maintain cellular structural and functional integrity. Degradation of phospholipids contributes to neuronal death in ischemic damage. In ischemia, enhanced hydrolysis of phospholipids is caused by increased activity of phospholipase A₂, which is induced by reactive oxygen

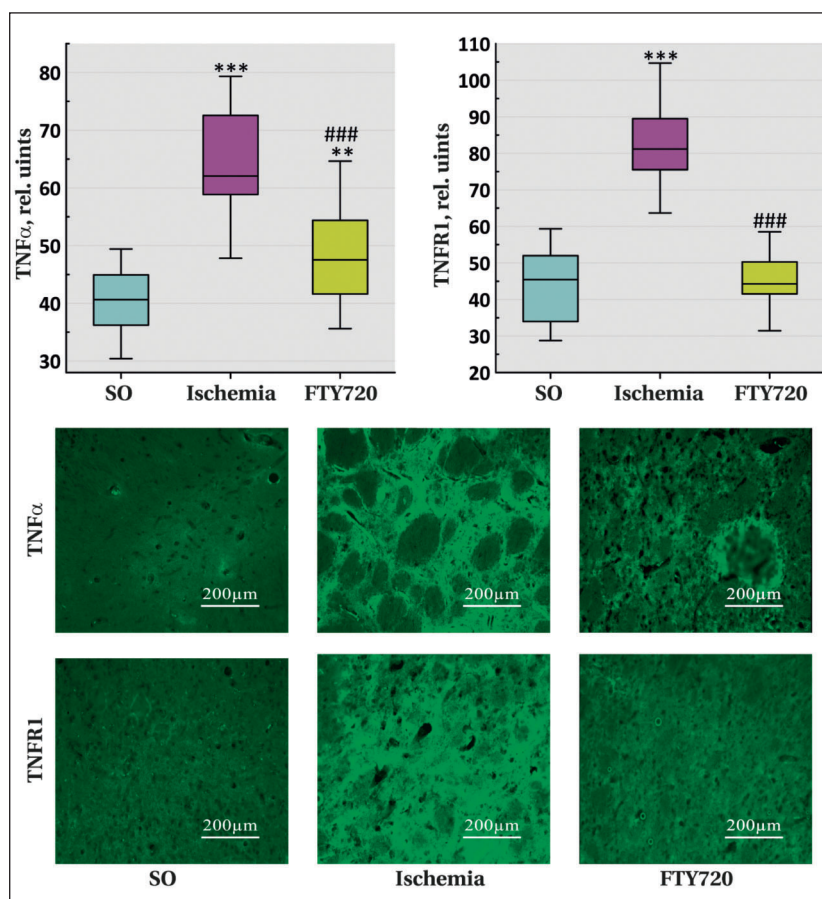


Fig 7. Dynamics of TNF α and TNFR-1 expression in the studied groups.

Note. ** — $p < 0.01$; *** — $p < 0.001$ — compared to SO animals; ### — $p < 0.001$ — compared to the group of animals with ischemia.

chemia model described by Reforgiato et al. (2016) demonstrated, that SPT activity was associated with increased levels of IL-6, IL-1 β , TNF- α , and increased production of reactive oxygen species. This suggests that inhibition of inflammatory responses by FTY720 may decrease SPT stimulation [22].

According to Henryk Jęśko et al. (2019) data obtained on the Alzheimer's disease model, FTY720 is able to suppress the expression of ceramide synthase mRNA [23]. Of interest is the lowered concentration of sphinganine, a precursor of sphinganine-1-phosphate, in cerebral ischemia. Established anti-ischemic effects of alkaloid nuciferin are accompanied by increasing levels of sphinganine-1-phosphate, while suppression of CerS activity leads to accumulation of sphinganine [24].

It has been reported that FTY720 reduces aS-Mase activity by causing proteolytic degradation of the enzyme complex, via a mechanism similar to that of tricyclic antidepressants [25]. In our study, FTY720 also reduced nSMase expression. Since the

direct effect of FTY720 on this enzyme has not been previously described, most likely this reduction is associated with a decrease in inflammatory infiltration and downregulated production of pro-inflammatory cytokines.

Conclusion

Acute cerebral ischemia in rats is associated with significant disruptions in lipid homeostasis, characterized by degradation of membrane phospholipids, accumulation of lysophospholipids, and activation of both the sphingomyelinase and «*de novo*» pathways of ceramide synthesis. These changes are accompanied by an increase in pro-inflammatory cytokine TNF α levels and expression of TNFR1, i. e., activation of the neuro-inflammatory response, and in entirety lead to development of severe neurological deficits and increased mortality.

Pretreatment with FTY720 produced a modifying effect on these pathological processes. This resulted in partial optimization of brain tissue phospholipid composition, a decrease in sphingomyelin degradation, and a reduction in ceramide accumulation. The latter came off inhibited expression of enzymes involved in catabolic and synthetic pathways of ceramide biosynthesis, such as acid and neutral sphingomyelinases, serine palmitoyl-transferase, and ceramide synthase.

Along with its effect on lipid metabolism, FTY720 reduces TNF α production and normalizes TNFR1 expression in ischemic brain tissue, down-regulating the pro-inflammatory response, thereby mediating a reduction in neurological deficits and an increase in animal survival during the acute phase of ischemic injury.

The data obtained indicate a significant role of lipid signaling disruptions, particularly ceramide-dependent mechanisms, in the pathogenesis of neuronal inflammation in acute cerebral ischemia. Modulation of sphingolipid metabolism and TNF α /TNFR1 signaling pathways by FTY720 can be considered as one of the key directions of neuroprotective strategy in ischemic brain damage.

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The Beneficial Effects of Krypton Inhalation Following Traumatic Brain Injury in Rats

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Summary

Traumatic brain injury (TBI) remains one of the leading causes of disability, and current approaches to neuroprotection have limited efficacy. The inert gas krypton is considered a promising neuroprotective agent; however, data on its effects in TBI and on components of the neurovascular unit (NVU) are limited.

Objective. To evaluate the neuroprotective potential of krypton in rats with traumatic brain injury (TBI) *in vivo* and in NVU cell cultures subjected to oxygen-glucose deprivation (OGD) *in vitro*.

Materials and Methods. The study included 48 Wistar rats divided into 3 groups: SO (sham operated), TBI (N_2/O_2 70/30 %), and TBI + iKr (Kr/O_2 70/30 %). A model of controlled open brain contusion injury was used. On day 14, we assessed neurological deficits (limb placing test, LPT), the extent of brain injury (T2-weighted MRI slices), morphological changes (hematoxylin-eosin staining), and the expression of GFAP and Caspase-3 (fluorescent immunohistochemistry, IHC). IL-1 β , IL-6, and TNF- α mRNA levels in the injury zone were determined by PCR. *In vitro* studies investigated the effect of krypton preconditioning (Kr/O_2 79/21 %, 24 h) on the survival of neuronal (SH-SY5Y), glial (C6), and vascular endothelial (Ea.Hy926) cells during OGD (4–6 h).

Results. A significant neurological deficit of 2.5 (2; 5.25) scores was determined by the TBI modeling accompanied by a large volume of brain damage of 33 (28; 39) mm³. Krypton inhalation led to a reduction in the lesion volume to 18 (15; 26) mm³ and accelerated the recovery of sensorimotor functions: starting on day 7, the indicators in the TBI + iKr group were statistically significantly better than in the TBI group, and by day 14, they approached the values of the SO (control) group. In the TBI + iKr group, IL-1 β and TNF- α levels in the affected hemisphere were nearly 50% lower than in the TBI group, while remaining higher than in the SO group; changes in IL-6 levels were insignificant. Histologically, less significant cerebral edema, spongiosis, and neuronal degeneration were observed in the TBI + iKr group. Immunohistochemical analysis revealed a trend toward more pronounced reactive gliosis (GFAP) with no differences in Caspase-3. *In vitro*, krypton preconditioning under OGD conditions did not improve the survival of neuronal, glial, and endothelial cells.

Conclusion. Krypton exerted significant neuroprotective effect in experimental TBI in rats, reducing neurological deficits, the extent of structural damage, and the severity of the inflammatory response. The absence of a protective effect in NVU cellular models underscores the essential role of systemic and intercellular interactions in the neuroprotective action of krypton and warrants further research into its mechanisms of action and dosing optimization.

Keywords: krypton; traumatic brain injury; *in vitro* neuroprotection; oxygen-glucose deprivation

Conflict of interest. The authors declare no conflict of interest.

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Introduction

The brain is one of the most protected yet most vulnerable organs. Brain injury may commonly result in catastrophic consequences, both for the individual and a whole society, placing a significant burden on the healthcare system, the social sector, and the economy [1]. In this regard, developing strategies aimed at preserving brain structure and

functions is one of the priorities of current critical care practice [2]. In this context, numerous strategies have been developed to minimize the consequences of brain injury, including the prevention and treatment of intracranial hypertension, ensuring adequate oxygen and nutrient delivery, and reducing energy deficit [3–7]. However, given current data on the pathogenesis of brain injuries associated with trau-

matic brain injury (TBI) and their contribution to the development of delayed neurocognitive disorders even with a favorable treatment outcome, the measures currently in use are insufficient [8].

In this regard, it seems timely to seek new strategies for preventing and mitigating the consequences of TBI that will complement existing approaches. One such strategy could be the use of so-called «neuroprotectants». This term currently refers to a group of substances capable of influencing key molecular mechanisms in the pathogenesis of neurotrauma and reducing the extent of damage to the neurovascular unit (NVU) [9].

Numerous neuroprotective agents have been synthesized and tested in the past, but none of them are sufficiently effective, and some turned to be even harmful during the acute phase of neurotrauma [10–12]. Unlike pharmacological agents, which generally have limited bioavailability and low ability to cross the blood-brain barrier, inert gases possess a number of properties that are attractive for neuroprotection [13–19]. They have extremely low chemical reactivity, enter and leave the body unchanged without forming metabolites, yet they possess relatively high biological activity.

For example, the inert gas xenon possesses anesthetic and analgesic properties and is used in anesthesiology and for the relief of pain [20–22]. Helium has long been used in the treatment of conditions associated with bronchial obstructive syndrome [23, 24], while argon has shown promise in preclinical studies as an organ protector in conditions associated with ischemia-hypoxia and «no-reflow» syndromes (i.e., failed or insufficient microcirculation recovery in tissues after removal of main blood stream occlusion). The primary focus of research in this area is neuroprotection [25, 26]. As a neuroprotective agent, argon offers a number of advantages: it targets key mechanisms in brain injury pathogenesis, does not produce sedative effect under normobaric conditions, and does not require specialized inhalation equipment in experimental setting [27, 28].

In reference to TBI, the evidence regarding argon's neuroprotective properties remains inconclusive. Depending on the injury model, the severity of the trauma, and the therapeutic window, the effect of argon ranges from moderate to completely absent [29, 30]. Krypton, on the other hand, remains the least studied substance («dark horse») in the context of organ protection.

In early *in vitro* studies, krypton did not demonstrate any cytoprotective effects. However, a distinct neuroprotective effect was observed in our recent study using a model of photochemically induced stroke. The effect of krypton on the activation of Nrf2 and Akt, the inactivation of GSK-3 β , and the suppression of NF- κ B expression has been

demonstrated [31, 32]. These proteins are involved in signaling pathways responsible for universal neuroprotective mechanisms, which may be sufficient to prevent secondary damage following TBI.

Objective: To evaluate the neuroprotective potential of krypton in rats with traumatic brain injury (TBI) *in vivo* and in NVU cell cultures subjected to OGD subjected to oxygen-glucose deprivation (OGD) *in vitro*.

Materials and Methods

The study was conducted in accordance with established national and international bioethical standards (Directive 2010/63/EU). The study protocol was approved by the Local Ethics Committee of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology No. 3/22/6 on December 14, 2022.

Study design: a prospective, randomized, controlled, experimental *in vivo* study with concurrent *in vitro* component.

Experimental animals. The study used 48 sexually mature male Wistar rats weighing 250–350 g. A total of 33 animals were included in the main protocol for simulating TBI. The animals were randomized into 3 groups: sham-operated (SO, $n=6$), TBI group ($n=11$), and TBI with krypton inhalation (TBI+iKr, $n=16$). Analysis of long-term neurological and morphological outcomes was performed on a per-protocol basis. It included only animals that completed the 14-day observation period without complications: SO group — $n=6$, TBI group — $n=8$, TBI+iKr group — $n=10$.

In addition, an independent subgroup ($n=15$) was formed and used exclusively for Western blotting without analysis of long-term outcomes. This subgroup included intact animals ($n=5$), animals from the TBI group ($n=5$), and animals from the TBI+iKr group ($n=5$).

Animals were assigned to groups using a random number generator (simple randomization). Sample size was calculated for the primary endpoint. The uneven distribution of animals among groups was stipulated by the study protocol. The larger size of the TBI+iKr group was due to the expected greater variability of the parameters and the need to ensure sufficient statistical power (80% at $\alpha=0.05$) to detect a potential neuroprotective effect. The number of animals in the SO group was minimized in accordance with the 3R principles (Reduction), since no significant variability in the studied parameters was expected in this group.

The researchers who conducted the neurological testing, MRI morphometry, and IHC analysis were blinded to the animals' group assignments.

The primary endpoint was the volume of brain damage as determined by MRI on day 14.

The secondary endpoints were: neurological deficits, levels of pro-inflammatory cytokines, the extent of GFAP and caspase-3 expression, and survival rates.

Traumatic brain injury modeling. Traumatic brain injury modeling was performed according to the method of controlled contusion injury to the exposed brain [33], which had previously been used in similar experiments. Main steps: after induction of anesthesia and shaving of the surgical field, a midline skin incision was made, and a trepanation hole (5 mm in diameter) was drilled above the sensorimotor cortex of the left hemisphere (2.5 mm lateral to the sagittal suture, 1.5 mm caudal to the bregma), and the injury was inflicted by an impact tip/striker placed over the dura mater. The wound was sutured, and the animal was placed under an infrared lamp with a thermoregulatory system to maintain a body temperature of $37 \pm 0.5^\circ\text{C}$. In the SO group, a similar trepanation was performed without applying a blow [33].

Krypton treatment. Inhalations were performed after the animals awoke from anesthesia (90 minutes after TBI induction), 24 hours after TBI, and 48 hours after TBI (time points D0, D1, and D2, respectively); each inhalation session lasted 120 minutes. A semi-closed exposure system was used to administer the krypton-oxygen gas mixture, consisting of a transparent sealed chamber (35 L) with inlet and outlet ports for gas delivery and removal, a recirculation loop with a cooler, a drying system (silica gel), and a CO_2 absorption system (sodium lime), as well as an exhalation valve and an APL valve for releasing excess pressure. Environmental parameters were monitored using temperature, humidity, and gas concentration (O_2 , Kr) sensors installed in the circuit. The gas supply was provided by mixtures from cylinders containing Kr/O_2 (70/30%) («KrypOxA 70/30», Akela-N LLC, Moscow, Russia) and N_2/O_2 (70/30%) («Nitrogen-Oxygen 70/30», «Akela-N» LLC, Moscow, Russia) mixtures via regulators and flow switches. All components were connected using standard rubber tubing and plastic fittings.

Assessment of neurological status. Neurological testing was conducted in accordance with the previously described protocol developed by De Ryck et al. [34] and subsequently modified by J. Jolkonen et al. [35], which has also been used in a number of previous studies [29, 31, 36].

Prior to testing, the animals were acclimated to handling daily for seven days to reduce their stress response to manipulation. The assessment consisted of a series of seven behavioral tasks designed to analyze the sensorimotor integration of the forelimbs and hindlimbs in response to visual, tactile, and proprioceptive stimuli [36].

MRI examination. On the 14th day following traumatic brain injury, the animals underwent magnetic resonance imaging. A high-field MRI scanner

with a magnetic field strength of 7 T and a gradient system up to 105 mT/m (BioSpec 70/30 model, Bruker, Germany) was used. Anesthesia was administered via inhalation with isoflurane at a concentration of 1.5–2%. The animals were secured in a special apparatus that provided stereotactic positioning and body temperature control [37].

A standard protocol for imaging brain structures in rodents, based on the acquisition of T2-weighted images and described in a previous publication [36], was applied.

Cytokine mRNA quantification by real-time PCR. The day after the final inhalation, decapitation was performed under anesthesia with 6% chloral hydrate at a dose of 300 mg/kg, followed by craniotomy and removal of the cerebral hemispheres. Cytokine mRNA levels were assessed by quantifying mRNA in rat brain for the pro-inflammatory cytokine genes IL-1 β , IL-6, and TNF- α using real-time PCR.

RNA was isolated using the RNeasy MiniKit (QIAGEN, USA) according to the manufacturer's protocol.

Primers for PCR were selected using the Beacon Designer software. The primer pairs used are shown in Table 1.

Real-time PCR was performed in a BioRad iCycler (USA) amplifier according to the protocol (Table 2) using a mixture with the following composition: Mixture B (intercalating dye Eva Green + recessive dye ROX + Taq Pol + 25 mM dNTP + buffer («Sintol»)) — 10 μL , primers (mixture of 10 μM forward and reverse primers — 0.5 μL , MQ — 9.5 μL , reverse transcription product — 5 μL).

Histological analysis. On the 14th day after TBI, the rats' brains were fixed in 10% formalin, embedded in paraffin, and sectioned into 4- μm -thick slices. The sections were deparaffinized in xylene and rehydrated in an ethanol gradient. Antigen retrieval was performed at high temperature in citrate buffer, pH 6.0 (Target Retrieval Solution, DAKO). The sections were cooled, washed three times in PBS (with buffer + Tween; Cell Marque) for 5 minutes each, and incubated in 3% H_2O_2 for 10 minutes to block endogenous peroxidase activity. To prevent nonspecific binding, Protein Block Serum-Free (Abcam) was used for 30 minutes. The sections were incubated at 37°C for 1 hour with primary antibodies against Caspase-3 (1 : 100; ab13847, Abcam) and GFAP (1:500; 556329, BD Pharmingen, San Jose, CA), diluted in Antibody Diluent (ab64211; Abcam). The sections were then washed twice in PBS, stained with hematoxylin, washed again, and mounted under a cover slip. Secondary antibodies from the Dako REAL EnVision Detection System (DAB) or the ImmPACT Vector Red Substrate Kit, Alkaline Phosphatase (AP) (SK-5105) were applied according to the instructions. The sections were dehydrated in 70%, 96%, and 100% ethanol and cleared twice in xylene. Images were acquired using a Nikon

Table 1. Sequences and key characteristics of the primers used for real-time PCR.

Gene	Animal	Primer sequences (forward and reverse), 5' – 3'	Annealing temperature, °C
18S rRNA	Rat	GACAGGATTGACAGATTGAT TTATCGGAATTAACCAGACAA	56
TNF- α	Rat	TTATCTACTCCCAGGTTCT TGGTATGAAATGGCAAATC	56
IL-1beta	Rat	AGAACATAAGCCAACAAGT ACACAGGACAGGTATAGAT	56
IL-6	Rat	TGATTGTATGAACAGCGATGATG CCAGAAGACCAGAGCAGATT TCACATCACAGTAGGAAGTT	56

Note. Reverse transcription was performed using the SuperScript III kit provided by Thermo Scientific (USA).

Table 2: Real-time PCR protocol.

Period	Time	Temperature, °C	Number of cycles
Initiation	5 min	95	1
Denaturation	1 min	95	45
Annealing	20 s	56	
Elongation (stage 1)	20 s	72	
Elongation (stage 2)	1 min	72	1
Obtaining the melting curve	30 s	Every 30 seconds, the temperature rises by 0.5 °C	45

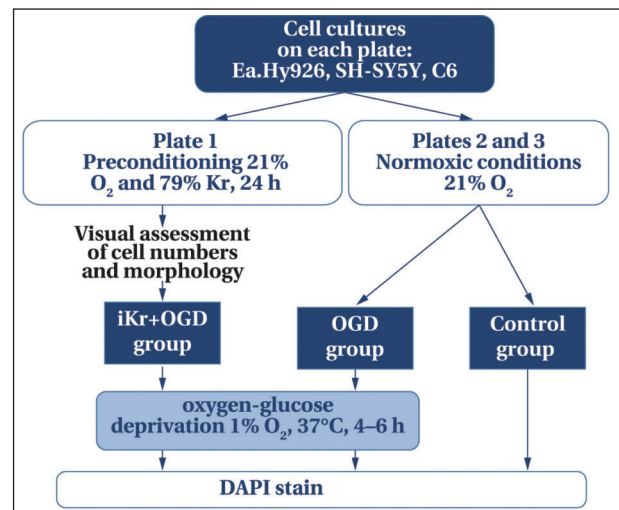
Note. For statistical analysis, the expression analysis results were normalized to 18S rRNA gene expression.

Eclipse Ni-e microscope and analyzed using NIS-Elements (Nikon) and ImageJ (NIH) software.

Modeling of the neurovascular unit. Inter-twined cultures of glial cells (C6), neuronal cells (SH-SY5Y), and vascular endothelial cells (Ea.Hy926) were thawed from stock solutions in a water bath at 37°C, then resuspended in complete culture medium (CCM). For C6 cells, the medium consisted of DMEM containing 1 g/L glucose + F12 (1 : 1) with 5% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotics (penicillin, streptomycin). For SH-SY5Y cells — DMEM containing 1 g/L glucose + F12 (1 : 1) with 20% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotics (penicillin, streptomycin). For Ea.Hy926 cells — DMEM containing 1 g/L glucose + F12 (1 : 1) with 5% fetal bovine serum (FBS), 1% L-glutamine, 2% hypoxanthine and thymidine (HT). Culture conditions in a CO₂ incubator: 5% CO₂, 37°C. Once a monolayer was formed, the cells were passaged using a 0.05% or 0.25% solution of trypsin with EDTA.

Preliminary experiment. All cell lines under study were seeded onto two 96-well plates: a control plate and a plate subjected to OGD for 4 hours. The percentage of cell death in all three cell lines following OGD treatment was determined by staining fixed cells with DAPI dye, according to the protocol described below. We assessed the need for shorter or longer OGD durations for the C6 and SH-SY5Y cultures and confirmed the subsequent experimental design involving pre-conditioning of the cultures with krypton.

Investigation of krypton protective properties in OGD. The experimental design is shown in Fig. 1. Three cell lines (Ea.Hy926, SH-SY5Y, and C6) were seeded into a single 96-well plate at a density of 10,000 cells per well. Three such plates were used:

**Fig. 1. Study design for NVU cells.**

1. iKr+OGD group (cell preconditioning with a krypton-oxygen gas mixture (Kr/O₂ 79/21%) (KripOx 79, «InertGas Medical» LLC, Moscow, Russia) for 24 hours, followed by OGD).

2. OGD group (normoxic incubator conditions with oxygen concentration maintained at 21% for 24 hours, followed by OGD).

3. Control group (normoxic incubator conditions with oxygen concentration maintained at 21% throughout the study, without OGD).

Once a monolayer was formed, Plate 1 was transferred to a desiccator filled with the appropriate gas mixture for 24 hours and left in a thermostat to maintain the temperature. Plate 2 was kept in the same incubator under standard normoxic conditions. In this case, the standard CCM for each culture was added to the cells, but only with DMEM and F12 media without sodium bicarbonate. After 24 hours,

the cells in the iKr+OGD and OGD groups were subjected to deprivation. Prior to OGD, a visual assessment was performed to determine whether exposure to the krypton gas mixture had affected cell number and morphology.

Plate 3 was kept in a thermostat under normoxic conditions; the standard culture medium for these cells was added to the cells, but using DMEM and F12 media without sodium bicarbonate.

Modeling of oxygen-glucose deprivation. To induce OGD, the cells were washed three times with DPBS. The cells in DPBS were then transferred to a multi-gas incubator with conditions of 1% O₂, 37°C for 4–6 hours. Subsequently, the cells were supplied with their standard culture medium, consisting of DMEM and F12 without sodium bicarbonate.

After the OGD, the cells were fixed with 4% formaldehyde at room temperature for 15 minutes, then stained with DAPI (300 nM) for 30 minutes. Imaging was performed using a Zeiss fluorescence microscope, followed by analysis in ImageJ.

Statistical analysis of the data was performed using SPSS Statistics software (version 27.0.1; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 8.0.1; GraphPad Software, Boston, MA, USA). The distribution of variables was tested for normality using the Shapiro–Wilk test. Statistical results were described as *Me* (Q1; Q3), where *Me* is the median, Q1 is the 25th percentile, and Q3 is the 75th percentile. If the distribution differed from the normal in at least one of the groups, nonparametric methods were used: the Mann–Whitney *U* test was applied to analyze differences between two independent samples; when comparing three or more groups, the Mann–Whitney *U* test was used, followed by correction for multiple comparisons using the Holm–Bonferroni method. For the analysis of repeated-measure data with a single factor, the Friedman test was used; when statistically significant differences were detected, a post-hoc analysis was performed using the Wilcoxon test and the Holm–Bonferroni correction for multiple comparisons. Differences between proportions in independent samples were assessed using Pearson's χ^2 test or Fisher's exact test if the expected frequencies were less than 10%. Differences were considered statistically significant at a *p*-value of < 0.05.

Results

Mortality and complications. During the 14-day observation period, 5 animals were withdrawn from the study due to reaching the predefined humane endpoint: 2 animals in the TBI group (18.2%) and 3 animals in the TBI+iKr group (18.8%). No statistically significant differences in the frequency of reaching the humane endpoint were found between the groups (*p*=0.677).

Postoperative inflammatory -necrotizing complications (abscesses) were observed in 1 animal in

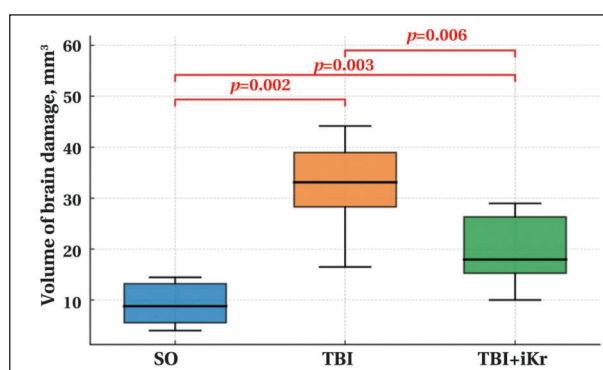


Fig. 2. Extent of brain injury as determined by MRI.

Note. For Figs. 2–4: the rectangles represent the interquartile range (Q1–Q3), the center lines represent the median, and the whiskers extend to 1.5 times the interquartile range. Red brackets indicate paired comparisons using the Mann–Whitney test with the Holm–Bonferroni correction; the exact adjusted *p*-values are shown above the brackets.

the TBI group (9.1%) and in 3 animals in the TBI+iKr group (18.8%). No statistically significant differences were found when comparing the incidence of infectious complications between the groups (*p*=0.645, Fisher's exact test).

Animals that reached the humane endpoint or developed infectious complications were excluded from further analysis of long-term outcomes.

MRI scan data. According to MRI findings on day 14, lesions were detected in all groups. A comparison of the three groups revealed statistically significant differences (Kruskal–Wallis test: *H*=15.98; *p*=0.0003). The volume of damage differed consistently and statistically significantly in all pairwise comparisons: the median (Q1; Q3) was 9 (6; 13) mm³ in the SO group, 33 (28; 39) mm³ in the TBI group, and 18 (15; 26) mm³ in the TBI+iKr group (Fig. 2).

Neurological examination. On the third day after simulated TBI, the median total score on the limb placement test (LPT) was 9 (5.5, 10.5) in the SO group, 2.5 (1.25, 4.75) in the TBI group, and 3.5 (2.75, 8.5) in the TBI+iKr group. A comparison of the three groups revealed statistically significant differences (Kruskal–Wallis test: *H*=9.62; *p*=0.008). Pairwise comparisons using the Mann–Whitney *U* test and Holm–Bonferroni correction showed a statistically significant decrease in indicators in the TBI group compared to the SO group (*p*=0.023). Differences between the TBI and TBI+iKr groups did not reach statistical significance (*p*=0.272).

On day 7, the values for LPT test were 12 (9.25; 13.3) in the SO group, 2.5 (2.5; 5.25) in the TBI group, and 9 (6; 10.3) in the TBI+iKr group. A general between-group analysis revealed statistically significant differences (*H*=10.85; *p*=0.004). Pairwise analysis demonstrated a statistically significant improvement in the TBI+iKr group compared to the

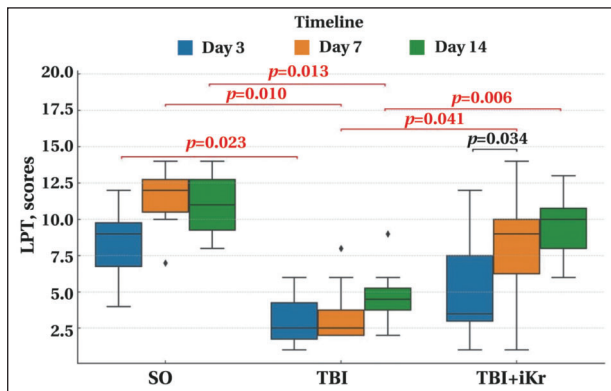


Fig. 3. Results of the limb placement test.

Note. Between-group p -values were determined using the Mann–Whitney U test, followed by Holm–Bonferroni correction for multiple comparisons (red brackets). Intra-group p -values were determined using the Friedman test with a post-hoc analysis by the Wilcoxon test and Holm–Bonferroni correction (black brackets).

TBI group ($p=0.027$), as well as statistically significant differences between the SO and TBI groups ($p=0.012$).

On day 14, the median LPT values were 11 (8.75; 13.3) in the SO group, 4.5 (3.25; 5.75) in the TBI group, and 10 (7.5; 11.3) in the TBI+iKr group. Intergroup differences remained statistically significant ($H=12.73$; $p=0.002$). Animals in the TBI+iKr group demonstrated statistically significantly higher scores compared to the TBI group ($p=0.006$), with values approaching those of the SO group (Fig. 3).

An intragroup analysis of changes in LPT scores was performed using the Friedman test. In the SO group, no statistically significant changes in total scores were observed during the observation period ($\chi^2=3.50$; $p=0.1738$). In the TBI group, the changes also did not reach statistical significance ($\chi^2=3.75$; $p=0.1534$). Statistically significant changes in the indicators were found in the TBI+iKr group, ($\chi^2=7.93$; $p=0.019$). A post hoc analysis using the Wilcoxon test and Holm–Bonferroni correction showed a statistically significant improvement between days 3 and 7 ($p_{\text{Holm}}=0.034$), whereas differences between days 7 and 14 did not reach statistical significance.

Therefore, krypton inhalation was associated with a statistically significant improvement in sensorimotor function beginning on the 7th day after injury, as well as significant positive intra-group trends, which were not observed in the TBI group.

Pro-inflammatory cytokines. Analysis of pro-inflammatory cytokines was performed on the third day following the induction of traumatic brain injury.

Analysis of IL-1 β revealed statistically significant differences between the groups (Kruskal–Wallis test: $H=12.28$; $p=0.002$). Simulation of traumatic brain injury revealed a statistically significant 2.8-fold increase in the concentration of the pro-inflammatory

cytokine IL-1 β in the injured cerebral hemisphere — up to 398 (346; 421) pg/g — compared to the intact brains of rats (141 (139; 154) pg/g ($p=0.024$). In the TBI+iKr group, the IL-1 β level was statistically significantly reduced by ~51% relative to the TBI group — to 194 (182; 211) pg/g ($p=0.024$), but remained higher than the values in the intact control ($p=0.048$).

Analysis of IL-6 concentrations did not reveal any statistically significant differences between groups ($H=2.96$; $p=0.228$). Traumatic brain injury resulted in a statistically insignificant increase in the concentration of the pro-inflammatory cytokine IL-6 to 122 (113; 134) pg/g in the TBI group compared to the intact brain at 101 (92; 121) pg/g ($p=0.234$). In the TBI+iKr group, IL-6 decreased to 102 (96; 112) pg/g, which also did not reach statistical significance compared to the TBI group ($p=0.747$).

Analysis of TNF- α levels revealed statistically significant differences between the groups ($H=12.52$; $p=0.002$). TBI led to a statistically significant 2.5-fold increase in TNF- α concentration in the rat brain — up to 205 (188; 243) pg/g versus 83 (77; 91) pg/g in the intact brain ($p=0.024$). In the TBI+iKr group, TNF- α concentration significantly decreased to 112 (112; 123) pg/g compared to the TBI group ($p=0.036$), but also remained higher than control values ($p=0.036$) (Fig. 4).

Histological examination. In the TBI group, histological analysis revealed marked structural abnormalities in both hemispheres. Thickening and partial detachment of the meninges, submeningeal hemorrhages, venous congestion with collapsed vessels, and marked perivascular edema were documented on the contralateral side. A cortical defect extending into the white matter was identified in the ipsilateral hemisphere, complemented by disruption of the meningeal integrity, plasma-filled vascular walls, glial infiltration in the penumbra zone, hemorrhages by diapedesis, and extensive spongiosis extending beyond the penumbra. In the penumbra and intact tissue, numerous dark neurons, shadow cells, and hypochromic neurons were detected.

In the TBI+iKr group, pathological changes were less obvious. The pia mater was thinner and adhered more tightly to the brain surface; submeningeal hemorrhages were detected only sporadically. Perivascular edema and spongiosis were moderately intense, and damaged neurons (dark cells, shadow cells) were encountered less frequently, especially outside the penumbra. Signs of perivascular and periglial edema persisted in the hippocampus, but to a lesser extent than in the TBI group (Fig. 5, a).

Inhalation of a krypton-oxygen mixture was associated with reduced edema severity, less extensive spongiosis, and a lower prevalence of degenerative neuronal changes outside the penumbra

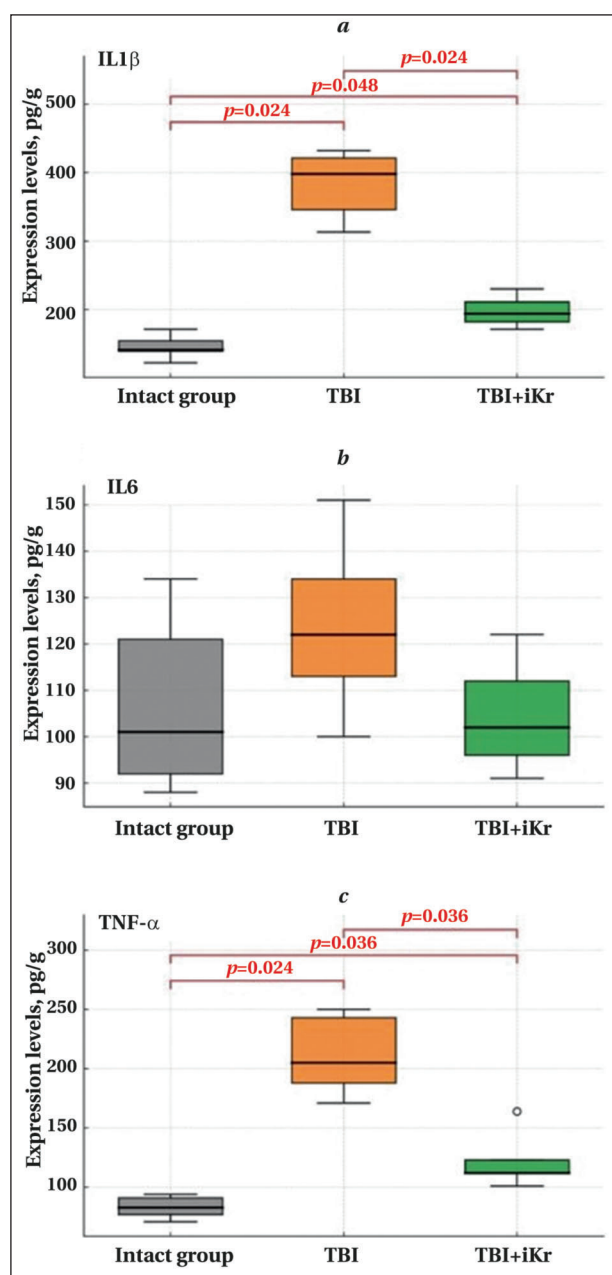


Fig. 4. Levels of pro-inflammatory cytokine expression in the experimental groups.

Note. Red brackets indicate paired comparisons using the Mann–Whitney *U* test, followed by correction for multiple comparisons using the Holm–Bonferroni method; the exact adjusted *p*-values corresponding to the statistical significance thresholds are shown above the brackets.

compared to the TBI group. These data indicate a potential neuroprotective effect of krypton, limiting the spread of structural brain damage following trauma.

Immunohistochemical examination of the brain. To assess the effect of krypton inhalation on cellular responses following traumatic brain injury, a quantitative comparison of GFAP (an astrocyte marker) and caspase-3 (an apoptosis marker) expression was performed in the ipsilateral and contralateral hemispheres of the brain.

Immunohistochemical analysis revealed a typical increase in GFAP expression in the ipsilateral hemisphere compared to the contralateral hemisphere, reflecting the development of gliosis in the injury zone in the TBI group. In the TBI+iKr group, GFAP expression activity in the ipsilateral zone was higher than in animals in the TBI group, with no differences on the contralateral side. Statistical analysis showed a trend toward increased GFAP levels in the TBI+iKr group ($p=0.086$) with no differences for Caspase-3 or contralateral values ($p=0.629$).

To clarify the nature of the changes, we calculated the differences between the sides (Δ GFAP and Δ Caspase-3), reflecting the degree of asymmetry in expression. The analysis showed that Δ GFAP in the TBI+iKr group was higher than in the TBI group ($p=0.086$), which confirmed the effect of krypton on enhancement of gliosis. Δ Caspase-3 values did not differ between the groups ($p>0.999$), indicating that krypton had no effect on the severity of apoptosis by the 14th day of observation. Thus, krypton inhalation following TBI was accompanied by increased gliosis, without exerting a significant effect on neuronal apoptotic activity (Fig. 5, *b*).

Results of the *in vitro* study. No statistically significant differences were found between the groups in the study of the protective effects of preconditioning with a krypton-oxygen breathing mixture on the survival of cell cultures comprising the NVU. The percentage of surviving neuronal cells (SH-SY5Y) after OGD relative to normoxia in the control group was 17.2 (14.6; 18.53)%, and in the krypton group, 16.2 (9.02; 20.1)% ($p=0.813$) (Fig. 6, *a*). The survival of cells of endothelial and glial origin also did not significantly improve under the influence of krypton, and the percentage of surviving cells was 77.6 (72.6; 89.9)% versus 69.8 (63.7; 84.8)% ($p=0.199$) in the Ea.Hy culture (Fig. 6, *b*) and 66.5 (57.9; 77.1)% versus 60.4 (57.6; 64.2)% ($p=0.160$) in the C6 culture (Fig. 6, *c*).

Discussion

The main finding of the study was the significant neuroprotective effect of krypton in an *in vivo* model of traumatic brain injury, as evidenced by both morphological data (MRI and histological examination) and functional tests (LPT). Furthermore, PCR data confirmed the anti-inflammatory nature of krypton's neuroprotective effect.

However, *in vitro* studies did not reveal any protective effects of krypton on any of the components of the NVU, which underscores the effectiveness of this inert gas only within the intact organism, but not in isolated cells.

The IHC study confirmed previous findings that the number of GFAP-positive cells in the injury zone increases in the delayed phase following in-

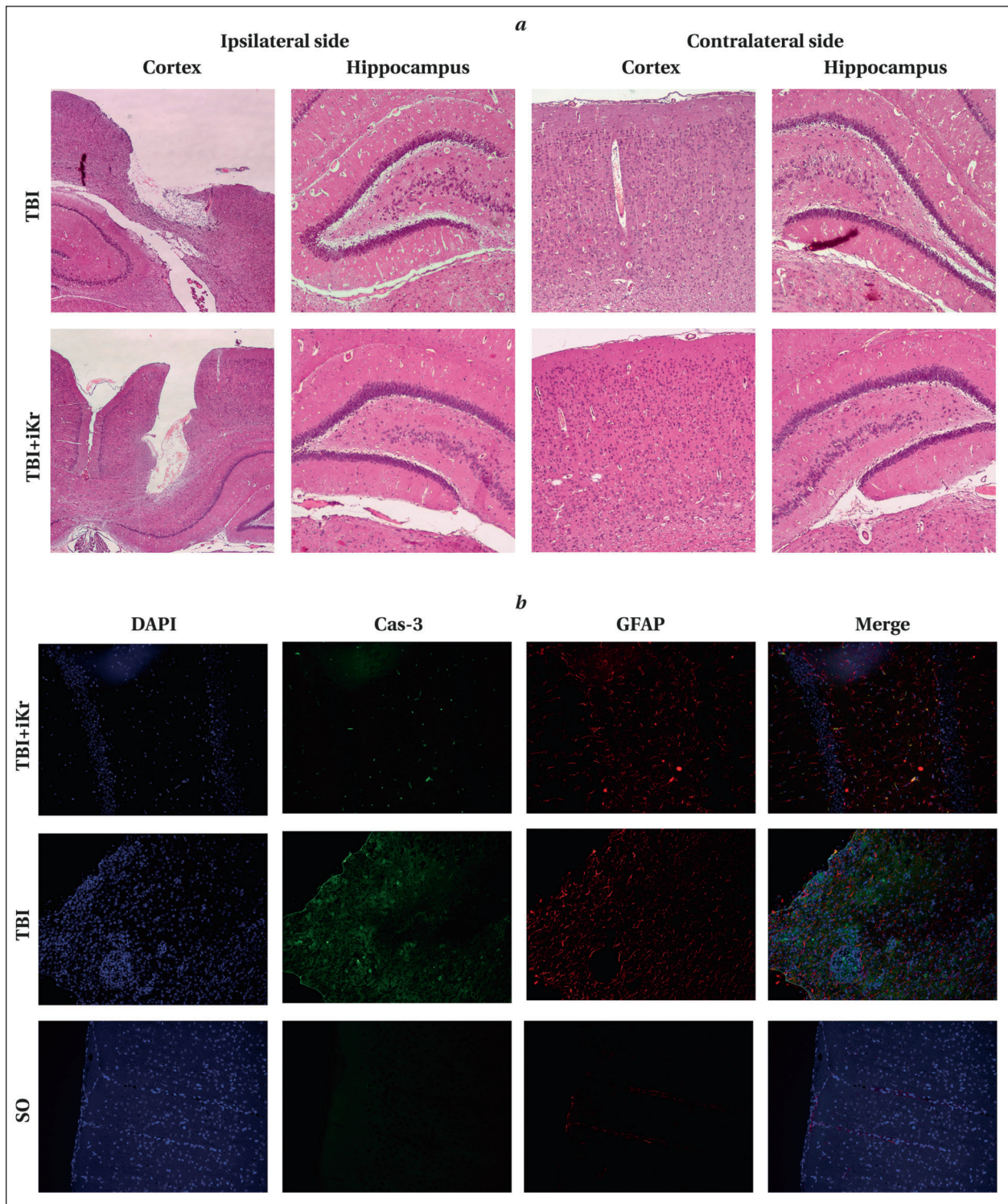


Fig. 5. Histological (a) and immunohistochemical (b) analysis of rat brain tissue following traumatic brain injury.

Note. *a* — Hematoxylin-eosin staining; *b* — Fluorescent immunohistochemical staining of the rat cerebral cortex. Blue — total number of DAPI-positive nuclei; Green — immune-labeled activated caspase-3 (Cas-3); Red color — immune-labeled astrocyte intermediate filaments (astrocytic glia, marker of reactive gliosis); Merge — overlay of all three channels. 20× magnification.

jury [40]. A higher number of GFAP-positive cells was also observed in the TBI+iKr group.

Although there is very little data on the neuroprotective effects of krypton [38, 40], given our previous studies [31, 41] using models of photochemically induced stroke, it can be assumed that the

neuroprotective effects of krypton also apply to traumatic brain injury. Of course, these results require further in-depth analysis to determine optimal concentrations and treatment regimens, as well as to clarify the molecular mechanisms underlying the observed effects. Nevertheless, the con-

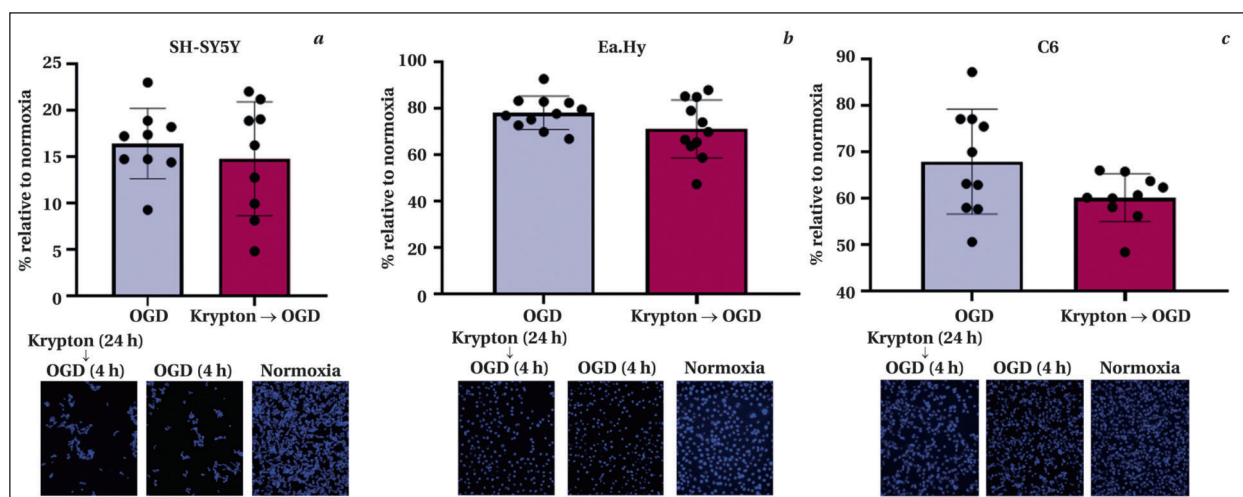


Fig. 6. Cells of neural origin (SH-SY5Y, *a*), vascular endothelial cells (Ea.Hy, *b*), and glial cells (C6, *c*).

sistent neuroprotective effect of krypton in models of brain injury of various etiologies may inspire researchers to expand the scope of studies on krypton-oxygen therapy.

It is worth noting that krypton and xenon not only share similar physicochemical properties but also exhibit similar biological effects, as demonstrated in *in vivo* studies [38, 42]. However, the results of *in vitro* studies [40, 43] show that, on isolated cells, krypton is capable not only of failing to exert a positive effect in cases of brain injury, but also of reducing the nerve cells resistance to hypoxia. Thus, in the absence of OGD, krypton reduced the regenerative capacity of intact cells ($p < 0.01$), which was not observed in studies with xenon or argon [40]. These data do not diminish krypton's neuroprotective potential but merely justify a differential approach to studying the molecular mechanisms of action of inert gases.

Any pathological process develops over time and progresses through successive stages, a fact that must be taken into account when planning therapeutic interventions. The efficacy of the same agent can vary significantly depending on the phase of injury: an intervention that has a pronounced

positive effect within the therapeutic window (e.g., thrombolysis in ischemic stroke) may, under other conditions, become neutral or even detrimental. This stage-dependent behavior likely explains the variability in the effects of inert gases when modeling the same pathology. Therefore, in the therapeutic application of inert gases, one should take into account both the characteristics of the agent itself and the nature and phases of the pathological process [44].

Conclusion

Krypton suppresses secondary damage in a rat model of traumatic brain injury, reduces neurological deficits during the first 14 days of observation, and reduces the volume of damage as determined by MRI. Beneficial effects of krypton gas are attributed to the suppression of the inflammatory process as demonstrated by a significant reduction in the mRNA levels of IL-1 β and TNF- α pro-inflammatory cytokines. However, no neuroprotective effect of krypton was observed *in vitro*. This duality and ambiguity of the results necessitate further investigation into the role of the whole organism in the manifestation of krypton's neuroprotective effects.

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The Use of Veno-Arterial Extracorporeal Membrane Oxygenation System for Refractory Circulatory Arrest Due to Accidental Hypothermia: a Clinical Case Report

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Summary

Thermal injuries caused by external cold exposure remain one of the most significant multidisciplinary challenges in critical care medicine. In cases of refractory circulatory arrest resulting from hypothermia, the use of circulatory support methods, particularly the veno-arterial extracorporeal membrane oxygenation (VA ECMO) system, becomes specially grounded and desirable.

Objective. To demonstrate the successful use of veno-arterial ECMO in a patient with severe accidental hypothermia complicated by refractory circulatory arrest during the prehospital phase.

Materials and Methods. We present the clinical case of a 42-year-old patient with severe generalized hypothermia (temperature 20.0°C), marked hemodynamic instability, and subsequent development of refractory ventricular fibrillation. Given the ineffectiveness of standard resuscitation measures during the prehospital phase, we initiated VA ECMO followed by extracorporeal rewarming and comprehensive intensive care.

Results. Following initiation of VA ECMO and controlled warming, restoration of cardiac rhythm and cardiorespiratory function was achieved. Weaning from VA ECMO was performed on the third day, and from respiratory support within the first two days of the hospital stay. On the 22nd day, the patient was discharged in a stable somatic condition without significant neurological deficits.

Conclusion. Early prehospital initiation of VA ECMO as a part of comprehensive intensive care may represent an effective treatment option for severe accidental hypothermia complicated by refractory circulatory arrest.

Keywords: hypothermia; out-of-hospital cardiac arrest; ventricular fibrillation; extracorporeal membrane oxygenation; warming

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Thermal injuries caused by external cold exposure remain one of the most significant multidisciplinary problems in critical care medicine [1]. Prolonged exposure to cold leads to deep-body cooling that reduces metabolic rates, i. e., drastically slows cellular metabolism, resulting in disruption of the central nervous, cardiovascular, and respiratory systems function, as well as other vital bodily functions. Concomitantly, these changes can lead to death [2].

In cases of refractory circulatory arrest resulting from hypothermia, the use of extracorporeal circulation support methods, particularly the veno-arterial extracorporeal membrane oxygenation (VA ECMO) system, becomes particularly important [3, 4]. When this technique is used, the 28-day survival rate of patients with hypothermic cardiac arrest (HCA) increases to 50% [5].

According to published evidence, VA ECMO is most effective in comorbid and elderly patients with hypothermia below 32°C, while in younger patients with unstable hemodynamics, i. e., arterial hypotension and cardiac arrhythmia, who are resistant to conventional intensive care VA ECMO is effective in hypothermia below 30°C. This corresponds to classes III (HT III) and IV (HT IV) on the Revised Swiss System (RSS) scale for patients with HCA who have HOPE scores ≥ 10 and ICE > 12 [6, 7].

Objective. To demonstrate the successful use of veno-arterial ECMO in a patient with severe accidental hypothermia complicated by refractory cardiac arrest during the prehospital phase.

Clinical case

Prehospital phase. Patient I., 42 years old, was found on the street by a passersby. Upon initial examination by the emergency medical team, the patient's condition was extremely critical: level of consciousness — 3 scores on the Glasgow Coma Scale, spontaneous respiration with RR 5 per minute, pulse in the major vessels — up to 20 beats per minute, blood pressure — 60/30 mmHg, ear canal temperature — 20.0 °C.

Intensive care was initiated during the prehospital phase, including cessation of further exposure to cold, orotracheal intubation with transition to mechanical ventilation, and infusion of warmed crystalloid solutions.

During preparation for evacuation, the patient developed ventricular fibrillation. Triple electrical impulse therapy as part of cardiopulmonary resuscitation (CPR) was ineffective; consequently, an emergency consulting anesthesiology and intensive care mobile team from A. S. Puchkov Station for Emergency and Urgent Medical Care in Moscow was dispatched to the scene to perform extracorporeal membrane oxygenation (ECMO)

Given the refractory circulatory arrest, femoro-femoral VA ECMO was initiated during the prehospital phase while mechanical chest compressions were ongoing. Canulation was performed via the right femoral vessels using a 21-Fr venous cannula and a 17-Fr arterial cannula. The flow rate was gradually increased to 2.5 L/min, and controlled warming was initiated at a rate of 0.5–1.5°C per hour. VA ECMO and vasopressor support with norepinephrine were ongoing during evacuation to the hospital; ventricular fibrillation persisted on the electrocardiogram (ECG) monitor (Fig. a). The patient was admitted to the intensive care unit (ICU) of the Regional Vascular Center (RVC) at the N. V. Sklifosovsky Research Institute of Emergency Medicine with a preliminary diagnosis of «Hypothermia. Clinical death».

Hospital phase. Upon admission to the hospital, the patient's condition remained extremely critical. He was unconscious; it was not possible to assess his neurological status at this stage. Hemodynamics were provided by VA ECMO support and with vasopressor therapy. Electrocardiogram monitor showed persisting ventricular fibrillation. Respiratory support was provided via mechanical ventilation. Body temperature, as indicated by the temperature sensor, was 21.6°C.

Examination of the skin in the areas of the knee joints, feet, and toes revealed areas of pale-cyanotic skin with decreased capillary response, consistent with localized cold injury (LCI). Echocardiography showed absence of effective cardiac contractions.

Laboratory blood tests demonstrated following results: creatine phosphokinase (CPK) was 248.25 U/L, D-dimer was 3.68 µg/mL, lactate was 18 mmol/L, and potassium was 2.94 mmol/L. Venous blood gas analysis revealed severe acidosis (pH — 7.049; BE — 16.1 mmol/L), hypercapnia (pCO₂ — 47.3 mm Hg), and a decrease in venous oxygen saturation to 65% with FiO₂ of 60%. Chemical and toxicological analysis confirmed the presence of ethanol in the blood and urine.

Based on the above data, the diagnosis of a general hypothermia, 3rd degree (severe), was established accompanied by a frostbite of the feet and knee joint regions (2nd–3rd degree), early reactive phase, post-clinical death condition, and alcohol intoxication.

Day 1 in the intensive care unit (ICU) of the Regional Vascular Center. 7 hours and 15 minutes after the onset of sustained ventricular fibrillation, after extracorporeal warming and upon reaching a temperature of 28°C in the ECMO circuit, defibrillation was performed, after which a conversion of the rhythm to atrial fibrillation was documented. Hemodynamics were maintained at a blood pressure of 87/50 mmHg using the VA ECMO system with a flow rate of 2.6 L/min and an infusion of norepi-

nephrine solution at a dose of 600 ng/kg/min. Due to the need for neuroprotection and synchronization with the mechanical ventilator, sedation with propofol was initiated. Continuous veno-venous hemodiafiltration was established to correct metabolic abnormalities.

The electrocardiogram showed atrial fibrillation with normosystole; the Osborne J-wave and QTc prolongation persisted (Fig. *b*). Echocardiography revealed preserved myocardial contractile function without areas of local hypokinesis.

Laboratory parameters showed persistent metabolic abnormalities: hyperlactatemia, hypokalemia, and an increase in creatine phosphokinase levels to 1484.18 U/L. Venous blood gas analysis revealed persistent signs of acidosis: pCO₂ — 30.0 mm Hg, pO₂ — 30.5 mm Hg, pH — 7.212, BE — 14.9 mmol/L. Controlled rewarming and correction of homeostatic disturbances were continued.

Day 2 in the intensive care unit (ICU) of the Regional Vascular Center. With ongoing controlled rewarming in 0.5–1.5°C increments and upon reaching a body temperature of 35.9°C, pharmacological sedation was discontinued to assess neurological status. The patient regained consciousness, was oriented to person, and followed commands. The mechanical ventilation settings were switched to assist-ventilation mode, and patient mobilization was initiated. Thirty-two hours after initiation of VA-ECMO, the body temperature reached 36.6°C.

Hemodynamics remained stable (BP 114/62 mmHg, HR 96–108 bpm) with the ongoing VA ECMO at a flow rate of 2.5 L/min and infusion of norepinephrine at a dose of 200 ng/kg/min, which allowed for the initiation of a gradual reduction in the extracorporeal circuit flow rate in 500 rpm/hour increments.

Over the course of the day, spontaneous conversion of atrial fibrillation to sinus rhythm was observed. Echocardiography revealed that satisfactory myocardial contractile function persisted, with no areas of local hypokinesis.

Laboratory blood tests showed an increase in C-reactive protein to 46.7 mg/L and in the total CPK fraction to 4280.80 U/L.

Continuation of continuous veno-venous hemodiafiltration.

Over the course of 2 days, after stabilization of respiratory function and normalization of the patient's blood gas composition were achieved, the patient was successfully weaned from mechanical ventilation, followed by tracheal extubation. During this same period, treatment was administered for concomitant complications, including local manifestations of cold injury and infectious-inflammatory processes in the respiratory organs.

Day 3 in the intensive care unit (ICU) of the Regional Vascular Center. The patient was fully conscious. Achieving of stabilized hemodynamics

and reduced need for vasopressor support substantiated a decision to disconnect the patient from the ECMO and remove the cannulas. Electrocardiography showed sinus rhythm; and over time, flattening of the Osborne wave and normalization of the QTc interval were documented (Fig. *c*).

Meanwhile, laboratory tests revealed persistent signs of severe myocardial injury with further increase of creatine phosphokinase (11,742.00 U/L) and myoglobin (2,162 ng/mL), which necessitated a 2-liter session of membrane plasma separation.

Thus, on the third day, after attaining normal cardiac rhythm and stabilized systemic hemodynamics, therapy was focused on discontinuing extracorporeal support and correcting secondary metabolic complications.

On day 5, the patient was fully alert, with no signs of neurological deficit. Hemodynamics remained stable without vasopressor support, and sinus rhythm was maintained. Breathing was spontaneous, with no signs of respiratory failure. According to echocardiography, satisfactory myocardial contractile function was maintained without areas of local hypokinesis. Laboratory parameters showed positive trends in terms of decreasing creatine phosphokinase levels. In a stabilized condition, the patient was transferred to the specialized (burns) unit to finalize treatment of localized cold injury sequelae.

On the 22nd day after admission, the patient was discharged from the hospital in satisfactory condition with no signs of significant neurological deficit.

Discussion

The acute form of general cold injury remains an underestimated form of thermal injury in our climate zone. A serious complication of severe hypothermia is cardiac arrest, the likelihood of which increases significantly when the body temperature drops to 30.0°C and becomes inevitable when it reaches 24.0°C [1].

There are published reports describing successful resuscitation from very deep accidental hypothermia of 13.7°C with circulatory arrest [8], and a case involving prolonged ventricular fibrillation lasting 6 hours and 45 minutes [9] with no subsequent neurological deficit.

Both cases required the use of extracorporeal therapies (assisted circulation and ECMO), therefore confirming their relevance in comprehensive management of severe general hypothermia. According to data from the multicenter prospective observational ICE-CRASH study [10], the VA ECMO system demonstrated the best survival rates in patients with hypothermic circulatory arrest, compared to other extracorporeal modalities. The advantages of VA ECMO also include a lower number of procedure-related complications and less aggressive anticoagulant support [11].



Fig. Electrocardiogram of patient I., 42 years old, with severe hypothermia.

a — during the provision of emergency medical care outside a medical facility; *b* — after rewarming and successful reversal of persistent ventricular fibrillation via electrical impulse therapy on the first day of admission to the ICU at the RVC; *c* — on the third day in the ICU at the RVC.

In the reported clinical case, comprehensive therapy using VA ECMO, initiated in the setting of prehospital care, was successful in a patient with severe general hypothermia complicated by persistent ventricular fibrillation lasting 7 hours and 15 minutes.

Russian-language published information on prehospital initiation of VA ECMO as part of resuscitation measures in patients with severe general hypothermia is very limited.

The rate of rewarming also remains a debated topic, although experimental studies and retrospective analyses have demonstrated the advantage of slow rewarming rates, which were associated with less neurological deficit, a less pronounced «afterdrop» effect (post-hypothermic temperature drop), as well as a positive effect on cardiac output [12–14].

The rewarming rate used (0.5–1.5°C per hour) was consistent with current recommendations for temperature management following circulatory arrest in adults [15] and was associated with a favorable neurological outcome, despite a prolonged period of sustained ventricular fibrillation.

Conclusion

This clinical case report of the successful treatment of severe accidental hypothermia, complicated by refractory circulatory arrest, demonstrates the effectiveness of comprehensive intensive care concept at all stages of medical care with early (pre-hospital) initiation of veno-arterial extracorporeal membrane oxygenation.

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Passive Euthanasia and the Withdrawal of Life-Sustaining Therapy in the Sociocultural Discourses of Western Countries and Russia: a Historical and Philosophical Analysis

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Summary

Passive euthanasia and the withdrawal of life-sustaining treatment (LST) in modern Russian practice are normatively indistinguishable, leading to ethical, legal, and clinical-practical conflicts for intensive care physicians and increasing the moral distress among healthcare staff.

The purpose of this research is to clarify the distinctions between active and passive euthanasia and the cessation of futile life-sustaining treatment, as well as to demonstrate why these distinctions are not taken into account in Russian legislation and professional discourse.

Materials and Methods. The study included foreign and Russian bioethical and legal texts. A qualitative analysis of the historiography was conducted, supplemented by a structural-thematic analysis of the philosophical dilemmas associated with the end of life.

Results. The analysis identified three key lines of demarcation: 1) between active killing and allowing death through the refusal of intervention; 2) between passive euthanasia and the ethical cessation of LST; 3) between the physician's intention to hasten death and the intention to remove medical obstacles to the natural process of dying while being willing to continue assistance if the patient survives. It was shown that the Russian legal norm, which effectively criminalizes any forms of cessation of medical treatment, has developed against the backdrop of insufficient reflection on these distinctions and leads to a discrepancy between normative language and actual clinical practice, where doctors are forced to document treatment provision but often omit or alter it in practice.

Conclusion. The integration into the Russian context of more nuanced criteria for assessing intention, causality, and futility of treatment, developed in Western bioethics, combined with consideration of local sociocultural contexts and religious traditions, is a necessary condition for developing a coherent end-of-life regulation model that reduces moral distress for physicians and enhances patient trust in medical decisions.

Keywords: *passive euthanasia; active euthanasia; futility of treatment; bioethics; history of medicine*

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Introduction

The prolonged existence of terminally ill patients in a coma has several socio-economic and medical consequences. Firstly, medical staff realize the futility of the interventions being performed, which provokes moral distress, exacerbates professional burnout, and leads to careless, formal care for such patients [1]. Secondly, these patients occupy scarce intensive care unit (ICU) beds, for which taxpayers spend money every day [2]. Moreover, the consideration of transferring more «promising» patients to the ICU becomes more complicated if beds are occupied. The bioethical principle of justice in the distribution of scarce medical resources requires justification for the presence of untreatable patients in intensive care units [3, 4]. Additionally, the associated complications, which are likely to lead to death, force the allocation of extra medical re-

sources to manage the condition of these patients. Finally, the inability of relatives to communicate with unresponsive patients and the state of uncertainty become an additional source of conflict with doctors [2]. Physicians express their internal conflict as follows: «We are obligated to continue full treatment, performing cardiopulmonary resuscitation (CPR) in the event of cardiac arrest — doing everything to prevent an unfavorable outcome» [2]. They understand the futility of their actions but must provide comprehensive medical intervention [5].

The medical community is unable to implement its understanding of treating such patients due to the prevailing ethical and legal perception of halting aggressive treatment for these patients via euthanasia. For example, Article 45 of Federal Law 323 makes no distinction not only between the cessation of futile treatment for incurable terminal

patients and passive euthanasia but also between active and passive euthanasia. Specifically, it clearly prohibits all forms of hastening death: «Medical professionals are prohibited from performing euthanasia, that is, hastening the death of a patient at their request through any actions (or inaction) or means, including the cessation of artificial life support measures» (Federal Law No. 323-FZ of November 21, 2011, Article 45) [6]. Thus, according to the law, all forms of terminating life-sustaining measures, even if they do not lead to the patient's recovery, are equally prohibited as either active or passive euthanasia [7]. A physician's decision to refrain from using burdensome and futile treatment to a dying patient may be prosecuted under several articles of the Criminal Code: murder (Criminal Code of the Russian Federation, Article 105) [8]; causing death by negligence due to improper performance of professional duties (Criminal Code of the Russian Federation, Article 109) [8]; and failure to provide assistance to a patient without justifiable reason by a person obligated to provide assistance by law or special rule, if this resulted in serious harm to health or death by negligence of the patient (Criminal Code of the Russian Federation, Article 124) [8].

On the other hand, Russian legislation recognizes the right of a patient to refuse unwanted treatment: «A citizen, one of the parents, or another legal representative of a person [...] has the right to refuse medical intervention or demand its cessation» (Federal Law No. 323-FZ of 2011, Article 20) [6]. Despite the seemingly legitimate opportunities for voluntary refusal of unnecessary treatment, paragraph 9 of Article 2 of the same law limits the implementation of such refusal, stating that «medical intervention without the consent of the person, one of their parents, or other legal representatives is permissible if the medical intervention is necessary for emergency indications to eliminate a threat to the life of a person» (Federal Law No. 323-FZ of 2011, Article 20, paragraph 9) [6]. Thus, this provision places exclusive responsibility on physicians for making decisions regarding the cessation of burdensome and medically futile interventions for terminal patients, including cardiopulmonary resuscitation. Similarly, Smolkova et al. (2013) provide arguments supporting the provisions of Russian legislation that stipulate criminal liability for medical professionals for actions or inactions classified as failure to provide assistance to a patient that led to the patient's death (Article 124 of the Criminal Code), even in cases of clinically ineffective and burdensome medical interventions [9]. Consequently, the legal system of the Russian Federation does not recognize the legitimacy of refusing therapeutic procedures deemed ineffective and burdensome. This normative position is reflected in academic discourse: researchers in academic publications often do not differentiate

between refusing clinically inappropriate treatment and passive euthanasia [9, 10].

Some attribute this negative legal attitude towards the cessation of treatment to religious institutions in the Russian Federation, particularly the Russian Orthodox Church (ROC) [11]. However, others believe that the ROC's position is, on the contrary, aligned with legislative norms and therefore does not differentiate between various forms of euthanasia in its ethical doctrine [12, 13]. The hypothesis of our study is that at the dawn of bioethics in Russia, the lack of proper bioethical analysis regarding what the cessation of life-sustaining therapy or other active or passive hastening of a patient's death entails led to contradictory provisions in Russian legislation. This assumption is supported by several studies [14–16].

In recent years, there has been a growing interest in euthanasia. In particular, the Russian Committee on Bioethics issued a statement in 2024 proposing a broad public discussion accompanied by educational activities. «It is necessary to support the preparation of popular educational programs on television, the publication of accessible literature explaining the meaning of euthanasia as a modern «medical practice», and the moral values that can determine a meaningful, morally justified, and politically effective response to this urgent issue [17]». Nevertheless, within the professional community of intensive care specialists, cautious perspectives on such initiatives have been expressed [2], despite that realities of medical practice revealing the shortcomings of legislation. Intensive care physicians are forced either to resort to deception to comply with the law while discontinuing futile and burdensome treatment for terminal patients or simply to ignore all the mentioned socioeconomic and ethical issues. Besides the moral distress in intensive care units, this may overall lead to a loss of trust in the medical profession in Russia [18]. To avoid (or at least mitigate) such shortcomings in the future, suggestions have been made in academic literature that Russian society should pay more attention to the bioethical analysis of what is involved in specific forms of medical decision-making related to the treatment of terminal patients [19]. Only legal norms based on such analysis can be sufficiently consistent to determine which actions should be prohibited, encouraged, or permitted, should such initiatives be undertaken in the future [20].

Materials and Methods

The study was interdisciplinary, incorporating both international and Russian bioethical and legal texts, including: *The Historiography of Ideas* by A. Lovejoy [21]; *The Specifics of Religious Discourse Structure* by A. M. Prilutsky, L. E. Andreeva [22]; *Discourse, Ethics, Public Health, Abortion, and Con-*

scientious Objection in Croatia by A. Borovečki, S. Babić-Bosanac [23]. A structural-thematic analysis was conducted on the philosophical dilemmas associated with the end of life.

Results

Medical interventions that may hasten death are influenced by various motivational factors. These serve as the basis for distinguishing between active euthanasia, passive euthanasia, and the refusal or withdrawal of medical treatment in cases of clinical ineffectiveness. Based on an analysis of relevant literature and scientific discussions regarding the care of terminal patients, the following distinctions are proposed: 1) between active euthanasia and the refusal of clinically inappropriate therapy, where in the former case the physician initiates a new pathological process leading to death, while in the latter case there is a cessation (or withholding) of medical intervention that obstructs the natural course of dying.

Furthermore, 2) a demarcation is established between passive euthanasia (in which a healthcare provider intentionally hastens the patient's death by refusing to apply or withdrawing life-sustaining treatment) and the refusal of clinically ineffective treatment (even if it could potentially lead to death). Also, 3) the significance of the physician's intention is emphasized, meaning a distinction is made regarding whether the physician, while applying, refusing, or abstaining from any medical intervention, intends to hasten death, foresees it, or simply considers the risk of a fatal outcome. These three distinctions ultimately allow for an ethical differentiation between passive euthanasia and the withdrawal of life-sustaining treatment. Following Macaulay [24], p. 136, life-sustaining treatment refers to types of care that are known or reasonably assumed to be capable of prolonging life, specifically: mechanical ventilation, hemodialysis, implantable pacemakers or cardioverter-defibrillators, infusion of vasoactive drugs, extracorporeal membrane oxygenation, and in some cases, antibiotic therapy. Although nutritional support is categorized by Macaulay as life-sustaining treatment, we consciously exclude it from our analysis due to the existing controversy in bioethical debates regarding whether it should be considered a medical intervention or simply the provision of feeding to the patient.

The difference between active euthanasia and the refusal of futile medical treatment.

In the case of *Vacco v. Quill* (1997), the plaintiffs argued that there is no fundamental moral distinction between killing a person and allowing them to die [25]. To support Timothy Quill's position, a group of philosophers submitted a statement noting that «Whether a doctor turns off a respirator in accordance with the patient's request or prescribes pills

that a patient may take when he is ready to kill himself, the doctor acts with the same intention: to help the patient die [26]». This kind of argument could also have been used in shaping Russian legislation regarding euthanasia, but with the opposite normative conclusion: all forms of intentional hastening of a patient's death have been deemed unlawful.

In addition, proponents of euthanasia argue that the distinction between killing and allowing to die does not hold independent moral significance. For instance, M. Tooley (1994) asserts that killing is considered a more serious act than consciously allowing someone to die, primarily due to external circumstances that intuitively lead us to perceive killing as more outrageous. He identifies several such circumstances: 1) motive — the intent to kill implies a more malevolent intention than the refusal to save, even if the latter is driven by laziness or negligence; 2) costs — preventing murder typically requires less risk or fewer public resources than maintaining life in the case of an incurable illness; 3) likelihood of death — in the case of murder, this likelihood is significantly higher than when opting not to intervene. In conclusion, Tooley argues that the difference in moral assessment between killing and allowing to die is influenced more by external empirical and psychological factors than by internal ethical considerations. To illustrate, he proposes a hypothetical situation involving two children, Mary and John, locked in a car with a single control button inside. If the button is pressed, Mary will be saved, and John will die; if the button is not pressed, Mary will sustain fatal injuries, and John will survive. Since there are no differences in motives, risks, and probabilities in this scenario, Tooley concludes that killing and allowing to die are morally equivalent [27].

Some authors have attempted to justify the equivalence between action and inaction, referencing the well-known example by J. Rachels of the «drowning children» [28, p. 112]. In both scenarios, two adults aim to inherit by eliminating their young nephews. The difference between the situations lies in the fact that in the first case, the adult actively drowns the child in the bathtub, while in the second, they simply do nothing, allowing the nephew to drown. According to J. Rachels, both individuals bear equal moral responsibility for the child's death, even though the distinction between direct action and passive inaction seems significant only at the level of describing their deeds.

W. Cartwright analyzes the difficulties of drawing a clear line between murder and the allowance of death, as well as the ease with which these two categories can be conflated (as in the examples of Tooley and Rachles). He argues that these are not homogeneous concepts, but rather «heterogeneous groupings containing a variety of types, such that

some of the types in one category are close to types in the other» [29]. Since individual instances from the group of murder and the group of allowing death sometimes appear similar or even overlap, it may give the impression that there is no moral difference between the groups. However, such individual coincidences cannot be generalized and unconditionally applied to all cases of murder and the allowance of death.

Such an intersection is possible because the act of killing can take both an active form — initiating a new lethal event — and a passive form — abstaining from actions that could prevent harm. Similarly, the allowance of death can occur not only passively, through non-interference in an already harmful event, but also actively — by removing obstacles that slow down the dying process. From this perspective, the case of Rachels, where an adult consciously refuses to save a drowning child, represents inaction that is effectively equivalent to murder, as the subject had the opportunity to prevent the death but chose not to. In the example of Tooley, on the other hand, pressing the button does not create a new lethal event, but merely redirects an already developing one, thereby demonstrating a visible moral equivalence between killing and allowing death.

The difference between passive euthanasia and the withdrawal of futile treatment.

Both passive euthanasia and the withdrawal (or non-initiation) of futile life-sustaining treatment share a common feature: a life-saving intervention is not undertaken. Therefore, they can both be seen as ways to «allow the patient to die». How can a moral line be drawn between them?

W. Cartwright emphasizes the importance of identifying the true cause of death by distinguishing between active deprivation of life and passive non-intervention: «Killing someone involves initiating a fatal causal sequence, whereas letting someone die involves allowing an existing fatal causal sequence to run its course. In the first case the cause of death is the person who initiated the fatal sequence, in the second it is the fatal sequence itself rather than the person who allowed it to continue» [29]. Thus, when it comes to passive non-intervention in an ongoing fatal process, the determining factor for establishing the true cause of death becomes the intent of the acting subject, rather than objective circumstances. A subject can be considered a valid cause of death if their refusal to provide assistance is accompanied by an intention to hasten the patient's death or is motivated by self-interest (for example, the desire to inherit from the dying person) when there is an objective possibility to change the course of events. In such a situation, the subject's intent becomes the decisive causal element that allows the lethal circumstances to come to fruition.

The opposite case is represented by a doctor who decides to cease medical intervention based on the clinical futility of such intervention and its burden on the patient. Here, there is no intention to kill, as the doctor's goal is to refrain from clinically irrelevant procedures rather than to bring about the patient's death. The criterion for the absence of intent to kill is the doctor's willingness to accept the possible situation in which the patient continues to live even after the cessation of life-sustaining treatment — this attitude indicates that the doctor did not aim to hasten the fatal outcome. Moreover, this willingness should be reflected in the fact that the doctor continues to treat such a patient.

Therefore, the decisive moral criterion is the physician's intention. In the case of active euthanasia, the medical professional creates «a new, lethal pathophysiological state with the specific intention in acting of thereby causing a person's death» [25]. This type of action is characterized by a simultaneous directed desire for the patient's death and the execution of a new act that directly leads to death. Hence, active euthanasia should be classified as a form of murder. A different ethical framework is observed in the refusal or cessation of treatment. Various motivations are possible here: if the physician consciously acts with the intent to end the patient's life, their action may also be regarded as murder. However, if medical intervention is discontinued (or not initiated) due to its excessive burden, adverse effects, or evident futility in the terminal stage of illness, then the physician is not killing but merely allowing the natural process of dying to conclude. In this case, the cause of death should be considered the illness, not the physician's inaction.

Thus, killing always remains morally unacceptable, while refraining from intervention or discontinuing treatment can be evaluated in different ways — either as an ethically wrong act (if there is an intention and goal to end the patient's life) or as permissible (if the intention is to acknowledge human mortality and the futility of therapy in hopeless cases). In other words, if a physician intends to bring about the patient's death by stopping life-sustaining treatment, they are guilty of passive euthanasia. The intention may include specific motives related, for example, to considerations of resource allocation (needing to free up a resuscitation bed for a more viable patient) or to personal interests. On the other hand, if their decision to refrain from such interventions or to discontinue them is motivated by concern for a patient with an incurable terminal illness, and the desire to avoid the misuse of medicine when the treatment is futile, then their care is focused on the patient and the art of medicine, allowing the natural dying process to take its course. Therefore, if, by forgoing treatment and allowing the dying process to proceed, the physician finds that the pa-

tient continues to live, and the physician is not disappointed but instead pleased with this outcome and thus provides medical support in another way, it indicates that the physician had no intention to kill the patient prior to discontinuing treatment.

In this section, two more points need to be discussed. The first is whether the initial intention can be mixed with other desires or beliefs related to the plan to end unbearable suffering. The second is how to assess the abuse of medicine, closely linked to the ineffectiveness of treatment.

Regarding the first question, it is important to note that assessing intentions is an extremely complex issue. The same physician may seek to alleviate a patient's unbearable suffering, especially in cases where palliative therapy is ineffective, while not wishing for the patient's death at all. It is crucial to distinguish between desires, beliefs, and actual intentions. An intention implies an internal stance of the physician, expressed in a specific plan of action. If a physician is motivated not only by compassion but also by the intention to end the patient's suffering through death, this intention will inevitably manifest in their subsequent actions. For instance, if the cessation of overly burdensome treatment does not lead to the patient's death, a physician with such an intention is likely to act in accordance with it — perhaps by withholding necessary medical support or deliberately taking other actions to hasten death. It is in such actions that the original intention is revealed. For example, if a patient begins to breathe independently after being removed from a ventilator, a physician who does not wish for the patient's death will continue to provide treatment in another form — without the use of mechanical ventilation. In contrast, a physician who consciously desires the patient's death may take active measures to end it upon noticing spontaneous breathing, in order to see the process through to completion. A telling example is the case of Karen Ann Quinlan. If Karen's relatives had aimed not to stop excessive treatment but to hasten her death, they would have felt frustrated to see that after being disconnected from the ventilator, she continued to breathe and live. However, they continued to care for her, and Quinlan lived for several more years. This is why their action was interpreted as «allowing her to die», rather than as an act of passive euthanasia [30].

Regarding the second question about defining the futility of treatment, we need to distinguish between physiological, probabilistic, and normative futility. Although the concept of futility carries some negative connotations [31], we will use this term to indicate that a futile treatment does not benefit the patient. An example may be aggressive treatment of terminally ill patients (for instance, cardiopulmonary resuscitation for patients dying from an incurable stage of cancer).

The treatment is considered physiologically useless if it cannot achieve its therapeutic goal in principle. The concept of probabilistic uselessness implies that achieving the goal is possible only in a certain percentage of cases. According to Macauley's observation, physicians differ in their assessment of what probability of success makes a treatment justifiable [24]. Some believe that any, even minimal, assumption of success excludes the possibility of the intervention being useless. Others, on the contrary, consider treatment useless if the likelihood of a positive outcome does not exceed 1–5%, meaning that it remains possible but extremely unlikely. Finally, there are researchers for whom even a 40–60% probability of success is insufficient to deem the treatment beneficial. Thus, the decision to forgo a useless intervention can have entirely different grounds and consequences for the patient. This vagueness in defining probabilistic uselessness poses serious ethical risks. If a treatment is only potentially useless but is nonetheless discontinued or not performed at all, it may lead to hastened death for the patient, which would be unethical. However, researchers emphasize that even cases of probable uselessness — more so than cases of physiological uselessness — inevitably depend on value judgments. [32]

Normative uselessness, based on the value beliefs of the physician, is even more complex. Here, the assessment of uselessness depends on the «intrinsic value of the goal» of the medical intervention [24]. An example of normative uselessness is the continuation of resuscitation treatment for a child with anencephaly [33].

The Importance of Intention.

We have already examined many aspects of a physician's intentions, such as the intention to hasten a patient's death versus the intention to allow them to die. This section deals with the principle of double effect, which helps distinguish between unethical and ethical intentions. A physician's intention deserves careful and consistent consideration. It is important to remember that determining the intentions of individuals, including physicians who disconnect life-sustaining treatment, is certainly impossible. However, this kind of distinction is made in murder investigations to identify mitigating or aggravating circumstances, so it is not entirely futile. The principle of double effect that we present as a criterion for the ethics of withdrawing futile treatment from terminally ill patients may contribute to its understanding.

The principle of double effect states that if an action is taken to achieve a positive, desirable effect but is accompanied by negative, undesirable risks, and if the likelihood of the positive effect outweighs the risk of the negative effect, then it is ethically permissible to undertake that risk. An example of

the double effect principle application is the prescription of opioids to a terminally ill patient whose death is inevitable when other means of alleviating unbearable pain are ineffective. In this case, if the negative effect of the opioids, respiratory depression, is not the intended goal but still occurs and ultimately hastens death, then the prescription of opioids is ethically justified according to the principle of double effect, as the negative effect was foreseen but not intended by the physician.

However, it is important to address the existing criticism of the principle of double effect, which is also related to the physician's intent definition. Opponents of the principle emphasize the significance of the purity of the physician's intentions and their responsibility for the consequences of treatment. Therefore, they argue that a physician cannot honestly claim that their sole intention is to aggressively treat the patient's pain, as they may be subconsciously or otherwise personally affected by the suffering of that patient, which could lead them to underestimate the risk of hastening the patient's death. Critics of the principle of double effect also contend that such additional intentions create a risk of indirect or unintended euthanasia.

However, this argument can be challenged. When following the principle of double effect, a doctor, even if they wish to shorten a life filled with suffering, still has the primary intention of alleviating unbearable symptoms. It is important to focus on the primary intention rather than its purity. Furthermore, as mentioned earlier, secondary intentions cannot even be called «intentions.» They pertain to the realm of desire — a compassionate desire to end the patient's intolerable suffering. The difference between intention and mere desire is that only the former involves a conscious plan of action.

Discussion

The proposal to use the physician's intent as the main criterion for distinguishing between passive euthanasia and ethical cessation of life-sustaining treatment raises two groups of issues: epistemological and institutional. The epistemological issue is related to the fact that a physician's internal motives are, by definition, inaccessible to direct observation, and can only be retrospectively reconstructed based on external actions and the sequence of clinical decisions, similar to how aggravating or mitigating circumstances are assessed in criminal proceedings. The institutional issue is that current Russian legal norms effectively encourage physicians either to mask their true motives (for example, through fictitious indications for continuing therapy) or to formally continue futile treatment to avoid the risk of prosecution under the Criminal Code of the Russian Federation.

In Western literature, criticism of the principle of double effect shows that an appeal to «purity of

intent» can be psychologically and practically untenable, as a physician simultaneously experiences compassion and is subjective to resource limitations and their own moral fatigue. However, an analysis that suggests distinguishing between intentions and desires and evaluating the specific plans of action reflected in behavior (for example, a physician's reaction to a patient's unexpected survival after therapy has been discontinued) allows the concept of intent to be transformed from a purely internal category into an ethical and legal criterion. In this sense, Russian bioethics could integrate more nuanced versions of the principle of double effect and related concepts of responsibility, without reducing them to either subjective «purity of heart» or a formal prohibition of any actions that increase the risk of death.

The development of the concept of «useless» treatment is particularly significant for the Russian discussion, as it is virtually absent in domestic literature or is replaced by morally charged terms such as «refusal of assistance» or «passive euthanasia». A detailed distinction between physiological, probabilistic, and normative uselessness, as documented in international sources, indicates that the decision to discontinue therapy inevitably contains a value component. However, this component can be structured and articulated in clinical guidelines rather than left to implicit individual assessments. In the Russian legal framework, where formal discussions of the uselessness of treatment are lacking, a physician finds themselves in a position where any limitation on therapy can potentially be interpreted as criminal negligence, despite the fact that selective decisions regarding the limitation of medical procedures are already being practiced in intensive care units.

This legal silence regarding the uselessness of treatment exacerbates the moral distress of medical personnel, increases the risk of professional burnout, and pushes them to circumvent the law in one way or another, which sometimes involves creating documentary fictions and implicitly changing treatment tactics. Thus, a vicious cycle is reproduced: the absence of a conceptual and legal language for describing ethical refusal of therapy leads to legal nihilism and a decrease in public trust in medical decisions regarding end-of-life care.

The demonstrated inconsistency between the legal prohibition of any forms of hastening death and real clinical dilemmas indicates a broader tension between the Western bioethics brought in and local sociocultural conditions. On one hand, Russia finds itself included in global bioethical discussions, where criteria for distinguishing between euthanasia and the withdrawal of futile treatment have already been established. On the other hand, these criteria are introduced against a backdrop of a lack of a stable tradition of public debate about end-of-

life issues and the strong role of state and religious leaders in shaping the normative agenda.

In this situation, appealing to traditional spiritual and moral values can play a dual role. On one hand, such appeal legitimizes resistance to the legalization of euthanasia and, in conditions of economic pressure and resource shortages, expresses concerns about viewing human life as a tool that individuals possess to achieve their life goals. On the other hand, without a clear distinction between euthanasia and the refusal of futile treatment, such discourse effectively reinforces the moral and legal obligation to preserve life at all costs, which contradicts both the basic principles of beneficence and non-maleficence and traditional understandings of the naturalness of death.

Conclusion

Thus, the following criteria can be proposed to distinguish between passive euthanasia and ethical withdrawal of life-sustaining treatment as used in Western sociocultural and legal contexts: 1) if a medical professional initiates a new pathological process that leads to the patient's death, they are killing the patient through active euthanasia; 2) if they refrain from saving the patient's life by withholding or withdrawing available treatment with the intention of hastening death, they are practicing passive euthanasia. In this case, the physician would be disappointed if the patient survives after the cessation of LST. On the other hand, 3) if a physician refuses or discontinues the treatment of a patient whom they consider to be dying because they believe that treatment is medically futile and burden-

some for the patient, the physician is not guilty of passive euthanasia; rather, they are simply removing a medical obstacle that interferes with the natural process of dying. In such cases, they will not be disappointed if the dying process unexpectedly reverses and the patient's life is prolonged, even for a short time.

Furthermore, the analysis conducted supports the thesis that ethical discussions should precede changes in legislation rather than serve as ideological justifications afterward. Otherwise, internally contradictory norms arise that simultaneously: 1) declare the patient's right to refuse treatment; 2) criminalize any cessation of life-sustaining treatment; 3) fail to provide physicians with a workable concept of futility. The use of the interdisciplinary field of bioethics in this context should focus not only on the theoretical delineation of categories but also on the analysis of the strategies that physicians actually employ to resolve conflicts in intensive care units, including their unofficial practices and moral arguments.

Only on the basis of such a «dual» analysis — philosophical-normative and empirical, grounded in practice — can a specific Russian model for end-of-life regulation be formed that, on one hand, takes into account local cultural and religious codes, and on the other, integrates international criteria of intention, causality, and futility of treatment. This would not only reduce the moral distress of medical personnel and increase patient trust but also establish a more sustainable trajectory for the development of domestic bioethics, moving beyond simple borrowing or rejection of foreign solutions.

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