www.reanimatology.com
ISSN 2411-7110 (online)

GENERAL REANIMATOLOGY

SCIENTIFIC-AND-PRACTICAL JOURNAL

ОБЩАЯ РЕАНИМАТОЛОГИЯ

научно-практический журнал

Volume 21 Том 21

№ 1

Dear Authors,

Thank you for choosing General Reanimatology for the publication of your research articles.

The Editorial Team of 2025 welcomes contributions on basic, clinical and experimental research related to the scope of the journal.

We encourage authors to include graphical summaries and highlights, as well as audio or video summaries (mp3, mp4, up to two minutes). These supplemental materials allow you to present a summary of the article, briefly comment on your findings, share your perspective on important issues, and ask the community questions about your research.

Supplemental audio and video files will be posted with your accepted article on the General Reanimatology website, www.reanimatology.com, after prepublication review.

The use of graphics, audio, and video formats to supplement your article will help broaden the readership, increase engagement with the material presented, improve understanding of the results, and ultimately increase citation rates.

We wish you success in your scientific and practical endeavors and look forward to continuing our fruitful collaboration!

Editorial Team,
General Reanimatology Journal

ISSN 1813-9779 (print) ISSN 2411-7110 (online)

GENERAL REANIMATOLOGY

OBSHCHAYA REANIMATOLOGIYA

Scientific-and-Practical Peer-Reviewed Journal
Since 2005

• Covers issues of critical care medicine
• Manuscripts in Russian and English are published free-of-charge
• Included in SCOPUS (since 2015), RINTs, RSCI, DOAJ, and other databases, as well as in the Official list of editions recommended for publication of dissertations (PhD, DSci) by the Russian Higher Attestation Commission

Registration certificate of the Journal «Obshchaya reanimatologiya» (General Reanimatology): ПИ № ФС77-18690, November 2, 2004, Federal Service for Supervision of Compliance with Legislation in the Sphere of Mass Communications and Protection of Cultural Heritage
Publication Frequency: 6 numbers per year.
Founder:

ОБЩАЯ РЕАНИМАТОЛОГИЯ

OBŜAÂ REANIMATOLOGIÂ

научно-практический рецензируемый журнал
Выходит с 2005 г.

• охватывает вопросы медицины критических состояний
• публикует рукописи на русском и английском языках бесплатно
• включен в базы данных SCOPUS (с 2015 г.), РИНЦ, RSCI, DOAJ и др. базы данных; Перечень изданий, рекомендованных ВАК для публикации результатов диссертационных работ

Свидетельство о регистрации: ПИ № ФС77-18690 от 02 ноября 2004 г. Печатное издание журнал «Общая реаниматология» зарегистрирован Федеральной службой по надзору за соблюдением законодательства в сфере массовых коммуникаций и охране культурного наследия.
Периодичность: 6 раз в год
Учредитель: © Фонд «Медицина критических состояний», Москва, Россия

Publisher:
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russia
Издатель:
Федеральный научно-клинический центр реаниматологии и реабилитологии (ФНКЦ РР), Москва, Россия

Supported by Russian Federation of Anesthesiologists and Reanimatologists
При поддержке Общероссийской общественной организации «Федерация анестезиологов и реаниматологов»

Artwork: Natalia V. Golubeva
Page-proof: Sergey V. Shishkov
Printing House:
Printed at LLC «Advanced Solutions». 19, Leninsky prospekt, build. 1, Moscow, 119071. www.aov.ru
Contacts:
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia.
Tel. +7-495-694-17-73.
E-mail: **journal_or@mail.ru;**
Web: **www.reanimatology.com**

Subscription:
Index 46338, refer to catalog of «Книга-Сервис»
Signed for printing: 06.03.2025

Оригинал-макет: Н. В. Голубева
Верстка: С. В. Шишков
Типография: отпечатано в ООО «Адвансед солюшнз». 119071, г. Москва, Ленинский пр-т, д. 19, стр. 1. www.aov.ru
Контакты с редакцией:
Россия, 107031, г. Москва, ул. Петровка, д. 25, стр. 2.
Тел.: +7-495-694-17-73.
E-mail: **journal_or@mail.ru;**
сайт: **www.reanimatology.com**

Подписка и распространение: индекс издания по каталогу «Книга-Сервис» — 46338.
Цена свободная
Подписано в печать: 06.03.2025

CONTENTS **СОДЕРЖАНИЕ**

https://doi.org/10.15360/1813-9779-2025-1-4-14

Prediction of Local Infectious and Inflammatory Complications After Reconstructive Surgery of Aorta

Oksana O. Grin[1]*, Natalia V. Beloborodova[2], Marina S. Grekova[1], Alisa K. Pautova[2]*, Eduard R. Charchyan[1], Boris A. Akselrod[1], Olga V. Dymova[1], Lyubov I. Rizun[1], Alexander A. Eremenko[1], Maxim A. Babaev[1]

[1] B.V. Petrovsky Russian Research Center for Surgery, 2 Abrikosov Lane, 119435 Moscow, Russia
[2] V. A. Negovsky Research Institute of General Reanimatology, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia

For citation: *Oksana O. Grin, Natalia V. Beloborodova, Marina S. Grekova, Alisa K. Pautova, Eduard R. Charchyan, Boris A. Akselrod, Olga V. Dymova, Lyubov I. Rizun, Alexander A. Eremenko, Maxim A. Babaev.* Prediction of Local Infectious and Inflammatory Complications After Reconstructive Surgery of Aorta. *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 4–14. https://doi.org/10.15360/1813-9779-2025-1-4-14 [In Russ. and Engl.]

***Correspondence to:** Oksana O. Grin, grin_oksana@mail.ru; Alisa K. Pautova, alicepau@mail.ru

Summary

Aim. To identify biomarkers for prediction and early diagnosis of infectious and inflammatory complications in patients after aortic surgery.

Materials and methods. The study included 57 patients who underwent surgical procedures on the aorta and its branches under cardiopulmonary bypass and myocardial ischemia. The cohort was divided into two groups: patients with an uneventful postoperative period (group 1, *N*=35) and patients with local infectious and inflammatory complications after surgery (group 2, *N*=22). Serum levels of procalcitonin (PCT), interleukins (IL-6 and IL-10), and aromatic microbial metabolites (AMM) were measured before surgery, upon admission, and six hours after admission to the ICU. On postoperative days 3 and 6 neutrophil, lymphocyte, and platelet counts were assessed, and neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated.

Results. There were no significant differences in sex, age, or comorbidities between groups 1 and 2. Patients in group 2 had a more severe intraoperative period and required a longer ICU stay. Predictive markers of complications included IL-6>143.35 pg/mL at ICU admission (sensitivity 42.9%, specificity 90.9%, AUC 0.789, 95% CI 0.669–0.909, $P<0.001$); PCT>0.12 ng/mL 6 hours after ICU admission (sensitivity 90.9%, specificity 54.3%, AUC 0.762, 95% CI 0.634–0.891, $P<0.001$); NLR >7.8 on postoperative day 3 (sensitivity 72.7%, specificity 68.6%, AUC 0.710, 95% CI 0.571–0.850, $P=0.003$); and △AMM (before and after surgery) >0.185 (sensitivity 77.3%, specificity 71.4%, AUC 0.780, 95% CI 0.651–0.909, $P<0.001$).

Conclusion. Values of IL-6, PCT, NLR, and AMM reflect different features of the inflammation and can be used for prediction and early diagnosis of infectious and inflammatory complications in cardiac surgery patients.

Keywords: infectious and inflammatory complications; biomarkers; interleukin-6; aromatic microbial metabolites; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; cardiac surgery

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

Information about the authors:
Oksana O. Grin: ORCID 0000-0003-1773-1291
Natalia V. Beloborodova: ORCID 0000-0003-2328-1610
Marina S. Grekova: ORCID 0000-0002-3010-2142
Alisa K. Pautova: ORCID 0000-0001-6470-0485
Eduard R. Charchyan: ORCID 0000-0002-0488-2560
Boris A. Akselrod: ORCID 0000-0002-4434-3123
Olga V. Dymova: ORCID 0000-0003-2008-6350
Lyubov I. Rizun: ORCID 0000-0002-9322-7858
Alexander A. Eremenko: ORCID 0000-0001-5809-8563
Maxim A. Babaev: ORCID 0000-0002-4288-3791

Introduction

Infections and inflammation account for 4.9% to 30.8% of all complications after cardiac surgery [1]. A large study analyzing the outcomes of more than 30.000 patients undergoing cardiac and vascular surgery reported the following incidences of infectious complications: urinary tract infection (2.8%), sepsis (2.2%), pneumonia (1.7%), surgical site infection (0.4%), and sternal wound infection (0.86%) [2]. Although these complications are relatively rare, they significantly prolong hospital stays, increase treatment costs [3], and reduce patients' quality of life. In some cases, these infections are diagnosed after discharge and may contribute to delayed adverse outcomes [4].

The inflammatory response plays a central role in the body's protective response to surgical stress. However, when this response is dysregulated — due to factors such as the patient's baseline

condition or specific characteristics of surgeries involving cardiopulmonary bypass (CPB) [5–8] — it can lead to tissue and organ damage, increasing the risk of infectious complications [9–14].

This study hypothesized that assessment of biomarker fluctuations related to immune, inflammatory, and metabolic homeostasis can differentiate between appropriate and dysregulated responses to surgical stress. This approach aims to establish a diagnostic panel for the prediction of infectious and inflammatory complications in the early postoperative period.

Materials and Methods

This prospective, minimally interventional study used data collected during the first phase of the scientific project «Microbiota Modulation» conducted at the Russian Scientific Center of Surgery named after Academician B. V. Petrovsky (Local Ethics Committee Meeting Protocol No. 7 dated April 15, 2021); registered at ClinicalTrials.com as NCT04921436 [15]. From 2021 to 2023, the study included patients (*N*=81) who underwent reconstructive aortic surgery under CPB and had myocardial ischemia (MI) without prolonged antibiotic prophylaxis.

Inclusion criteria:

— Age between 18 and 75 years;

— Reconstructive aortic surgery performed by the same surgical and anesthesia team;

— The patient's voluntary informed consent to participate in the study.

Exclusion criteria:

— Loss to follow-up due to patient transfer to another hospital (*N*=2);

— Development of postoperative complications such as bleeding in the early postoperative period requiring reoperation, severe hemodynamic instability, acute cerebrovascular events, and others (*N*=22) (Fig. 1).

Data from 57 patients were analyzed, including 43 males and 14 females (24.6%). The median age of the participants was 57 years (IQR: 46–64), with a Charlson Comorbidity Index (CCI) score of 4 (IQR: 2–5). The median duration of CPB was 124 minutes (IQR: 99.5–161), and the median duration of MI was 97 minutes interquartile range (IQR: 67.5–120.5). All patients were monitored in the intensive care unit (ICU) for at least 24 hours postoperatively.

The primary endpoint of the study was the occurrence of local infectious and inflammatory complications (e. g., pneumonia, surgical site infection) during the postoperative period, collectively referred to as «complications». According to national clinical guidelines [16], the diagnosis of pneumonia was made on the basis of the presence of new focal infiltrative changes in the lungs observed on radiological imaging, combined with at least two clinical and laboratory criteria:

— Acute fever of 38.0°C or higher;

— Cough with sputum;

— Physical signs such as focal crepitus/crackles, bronchial breath sounds, dullness on percussion;

— Leukocytosis $> 10 \times 10^9$/L and/or neutrophilic shift to the left > 10%.

The diagnosis of surgical site infection was made in collaboration with the surgeons based on inspection of the postoperative wound at dressing changes, including the results of microbiologic analysis of wound exudate.

Secondary endpoints included ICU length of stay and total hospital length of stay.

Group 1 included patients with an uncomplicated postoperative period (*N*=35), while group 2 included patients with complications (*N*=22). Of the patients in group 2, 19 (86.4%) were diagnosed with hospital-acquired pneumonia and 2 presented with purulent tracheobronchitis. Surgical site infections were observed in 3 patients (13.6%).

Blood samples for biomarker assessment were collected both pre- and post-operatively: immediately upon

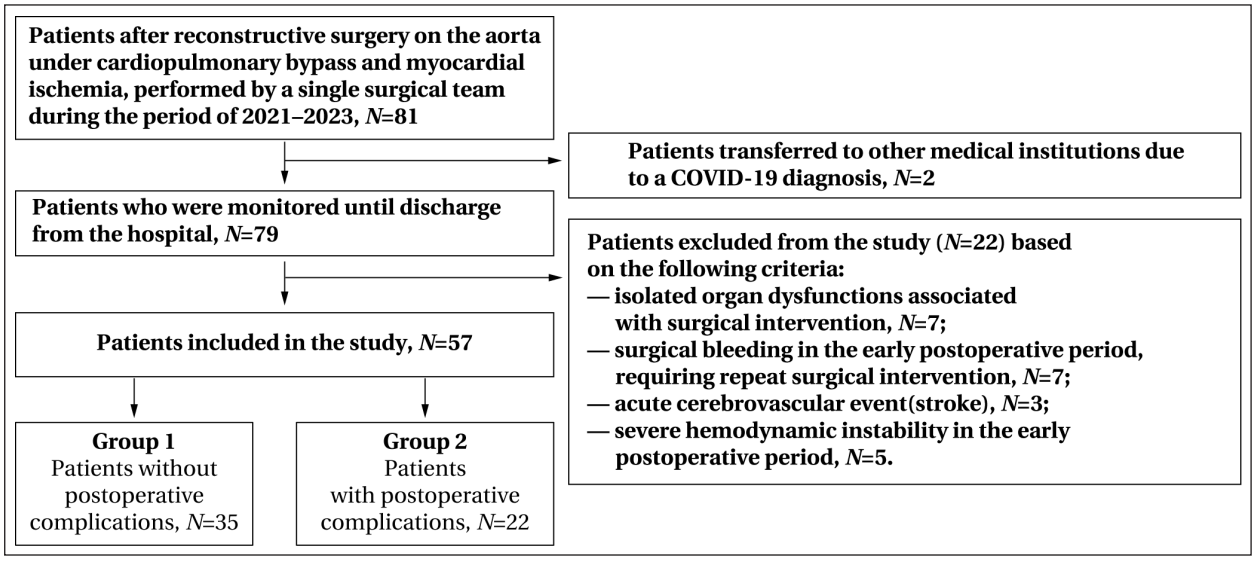

Fig. 1. Flowchart of patient inclusion in the study.

admission to the ICU and 6 hours after ICU admission. Levels of procalcitonin (PCT, cutoff < 0.05 ng/mL) were measured by immunochemistry, while tumor necrosis factor-alpha (TNF-α, cutoff < 50 pg/mL) and interleukins (IL-6 < 7 pg/mL and IL-10 < 9.1 pg/mL) were measured by enzyme-linked immunosorbent assay (ELISA).

At the same time points, serum samples were analyzed for clinically relevant aromatic microbial metabolites (AMM), including 2-hydroxy-3-phenylpropionic (phenyllactic), 3-(4-hydroxyphenyl)-2-hydroxypropionic (p-hydroxyphenyllactic), and 4-hydroxyphenylacetic (p-hydroxyphenylacetic, or p-HPAA) acids. AMM levels were determined in μM using gas chromatography-mass spectrometry after liquid-liquid extraction and derivatization. These metabolites were then summed to calculate an integral indicator: total aromatic microbial metabolites (∑AMM).

Dynamic changes in the parameters were calculated using the formula:

$$\triangle \text{Parameter} = 100\% \times \frac{\text{Value of the repeated measurement} - \text{Value of the previous measurement}}{\text{Value of the previous measurement}}$$

On postoperative days 3 and 6, a complete venous blood count was performed to assess neutrophil, lymphocyte, and platelet counts. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated from these values.

Microsoft Excel was used to construct the database and create graphical materials. Statistical data analysis was performed using IBM SPSS Statistics 26 software. The distribution pattern for each quantitative variable was determined using the Shapiro–Wilk test. All quantitative data with non-normal distribution were presented as median *(Q2)* and IQR *(Q1; Q3)*. Differences between two independent samples were assessed using the Mann–Whitney test, differences between paired samples were evaluated using the Wilcoxon test, and differences between three independent samples were analyzed using the Kruskal–Wallis test. Correlation analysis was performed by calculating Spearman's correlation coefficient (*R*). To evaluate variables as predictors, ROC analysis was performed and all results were accumulated in the Appendix (Table A2). In all cases, statistical analysis results were considered significant at $P<0.05$ (two-tailed *P*-value).

Results and Discussion

The age, sex, and comorbidity profile of the patients did not differ significantly between the groups being compared (Table 1). Although males predominated in both groups, male sex was not identified as a risk factor for developing complications (OR 1.8, 95% CI 0.49–6.66).

There were no fatal outcomes during the study. Comparison of parameters showed that group 2 patients experienced longer duration of CPB and MI, and significantly higher intraoperative and postoperative blood loss. In addition, this group required more intensive monitoring in the ICU during the early postoperative period and a longer overall hospital stay (Table 1).

The complete list of laboratory parameters studied is provided in the Appendix (Table A1). In the first step of data analysis, biomarkers and calculated parameters that could potentially serve as predictors of infectious complications were selected. Subsequently, only those parameters with statistically significant differences between groups 1 and 2 in at least one of the steps were considered (Table 2).

Correlation analysis was performed both between the studied markers and between the markers and parameters such as ICU length of stay, blood loss volume, CPB duration, MI duration, CCI, and patient age. A significant positive correlation was found between IL-6 levels on ICU admission immediately after surgery and PCT levels on postoperative day 6 ($R=0.543$, $P<0.005$). Further analysis results are shown in the Appendix (Tables A3, A4).

It was demonstrated that patients with complicated and uncomplicated postoperative course differ in the nature and intensity of the inflammatory response to surgical stress, allowing the development of a diagnostic biomarker panel. This panel could help to assess the likelihood of complications and contribute to their early diagnosis:

- A significant increase in IL-6 levels on admission to the ICU above 143.35 pg/mL (sensitivity 42.9% and specificity 90.9%) may indicate the de-

Table 1. Demographic characteristics, comorbidities, and features of the perioperative period in the study groups.

Parameter	Values in groups		*P*-value
	Group 1, *N*=35	Group 2, *N*=22	
Demographic characteristics and comorbidities			
Age, years	58 (45; 63)	53.5 (48; 65.75)	0.634
CCI, points	3 (2; 5)	4 (2; 5)	0.451
Proportion of men in the group, %	72%	82%	
Features of intra- and postoperative periods			
CPB, minutes	112 (73; 141.5)	161 (136; 206.5)	<0.001
MI, minutes	84.5 (53.75; 104.5)	122.5 (90.5; 151.75)	0.002
Intraoperative blood loss, mL	800 (600; 900)	1000 (725; 1875)	0.011
Blood loss in drains within the first 24 hours, mL	200 (140; 300)	325 (207.5; 500)	0.005
Follow-up time after surgery in ICU, days	1 (1; 1)	3.5 (2; 5)	<0.001
Follow-up time after surgery in the ward, days	7 (6; 8)	10 (7.25; 14.75)	0.001

Table 2. Levels of markers and calculated parameters in the study groups.

Parameter	Values in groups		*P* value
	Group 1, *N*=35	Group 2, *N*=22	
Before surgery			
p-HPAA, μM	0.4 (0.3; 0.6)	0.6 (0.4; 0.8)	0.078
∑AMM, μM	2.3 (1.9; 3.1)	2.4 (2.0; 3.2)	0.876
TNF-α, pg/L	6.20 (5.35; 7.05)	7.00 (5.00; 8.30)	0.349
IL-6, pg/mL	2.25 (1.50; 7.33)	7.70 (2.90; 17.20)	0.058
IL-10, pg/mL	5.00 (5.00: 5.00)	5.00 (5.00: 5.00)	0.281
IL-6/ IL-10 ratio	0.45 (0.30; 0.99)	0.90 (0.30; 2.42)	0.207
PCT, ng/mL	0.02 (0.02; 0.02)	0.02 (0.02; 0.04)	0.657
Upon admission to ICU			
p-HPAA, μM	0.3 (0.3; 0.6)	0.6 (0.4; 1.1)	0.018
∑AMM, μM	2.3 (1.9; 3.3)	3.2 (2.6; 4.5)	0.008
ΔAMM (before vs, after surgery), %	7 (–1; 21)	31 (19; 75)	<0.001
TNF-α, pg/L	6.10 (5.43; 7.98)	6.80 (5.40; 7.80)	0.527
IL-6, pg/mL	51.30 (34.10; 85.00)	125.50 (66.30; 218.30)	<0.001
IL-10, pg/mL	263.50 (135.00; 603.50)	253.00 (114.00; 773.00)	0.979
IL-6/ IL-10 ratio	0.17 (0.08; 0.56)	0.38 (0.18; 1.27)	0.031
6 hours after ICU admission			
p-HPAA, μM	0.3 (0.2; 0.4)	0.5 (0.3; 1.3)	0.001
∑AMM, μM	2.7 (2.3; 3.9)	4.1 (3.0; 5.6)	0.010
ΔAMM (before surgery vs, 6 hours after surgery), %	10 (–5; 32)	12 (–5; 23)	0.889
ΔAMM (before surgery vs, 6 hours after surgery), %	24 (3; 46)	49 (31; 116)	0.007
TNF-α, pg/L	5.20 (4.43; 6.93)	7.40 (5.20; 8.20)	0.030
IL-6, pg/mL	78.85 (51.60; 102.95)	86.20 (53.60; 135.60)	0.716
IL-10, pg/mL	15.70 (5.18; 46.48)	66.60 (6.70; 138.00)	0.068
IL-6/ IL-10 ratio	4.51 (1.71; 13.67)	0.96 (0.65; 15.18)	0.042
PCT, ng/mL	0.11 (0.06; 0.25)	0.42 (0.18; 0.87)	0.001
3rd day post-op			
Neutrophil count, 10^3 cells/μL	8.60 (6.50; 11.65)	8.85 (7.10; 10.00)	0.544
Lymphocyte count, 10^3 cells/μL	1.40 (1.05; 1.85)	1.00 (0.83; 1.18)	0.004
NLR	6.14 (4.05; 8.66)	9.52 (7.36; 12.14)	0.008
6th day post-op			
Neutrophil count, 10^3 cells/μL	6.50 (5.00; 8.35)	8.65 (6.43; 9.98)	0.017
Lymphocyte count, 10^3 cells/μL	1.80 (1.25; 2.05)	1.20 (1.00; 1.65)	0.028
NLR	3.78 (2.89; 4.96)	6.09 (4.50; 8.28)	0.002

velopment of a dysregulated hyperinflammatory response to surgical stress.

- PCT levels > 0.12 ng/mL six hours after ICU admission, with relatively high sensitivity (90.9%) but low specificity (54.3%), may identify patients at increased risk of complications.
- The blood level of an aromatic microbial metabolite p-HPAA > 1.1 μM on ICU admission and six hours later shows the highest specificity (94.3% and 94.1%, respectively) but low sensitivity (27.3% and 36.4%, respectively) for predicting the development of infectious inflammation.
- ∑AMM (sum of aromatic microbial metabolites), both as an absolute value on ICU admission and its changes pre- and postoperatively, as well as changes from preoperative levels to six hours after ICU admission, shows the highest sensitivity (81.8%, 77.3%, and 72.7%, respectively) and specificity (60%, 71.4%, and 71.4%, respectively) for predicting the development of infectious inflammation.
- An NLR > 7.8 on the third day after ICU admission has a predictive value for the development of infectious complications with a sensitivity of 72.7% and a specificity of 68.6%. By day 6 after ICU admission, NLR > 5.4 shows lower sensitivity (68.2%) but higher specificity (80%), suggesting that NLR at day 6 is a better marker of ongoing inflammation.

Patients in group 2 had longer CPB duration, greater blood loss and a marked pro-inflammatory response immediately after surgery. This response subsequently shifted to an immunosuppressive trajectory of the systemic inflammatory response, predisposing them to complications.

In contrast, patients in group 1 showed a less pronounced increase in IL-6 levels after surgery, although a slight upward trend was observed six hours postoperatively. The ratio of IL-6 to IL-10 was higher in group 1 than in group 2, reflecting preserved protective inflammatory mechanisms. By day 6, NLR levels returned to values typical of a relatively healthy population in group 1, whereas they remained elevated in patients with complications (Fig. 3).

Classic clinical signs of infection and biomarker levels are not always sufficiently informative; for example, fever in cardiac surgery patients may have both infectious and non-infectious origins.

In a prospective study conducted in an intensive care unit, changes in C-reactive protein (CRP) levels were not shown to be a reliable marker of infection, in contrast to elevated body temperature and leuko-

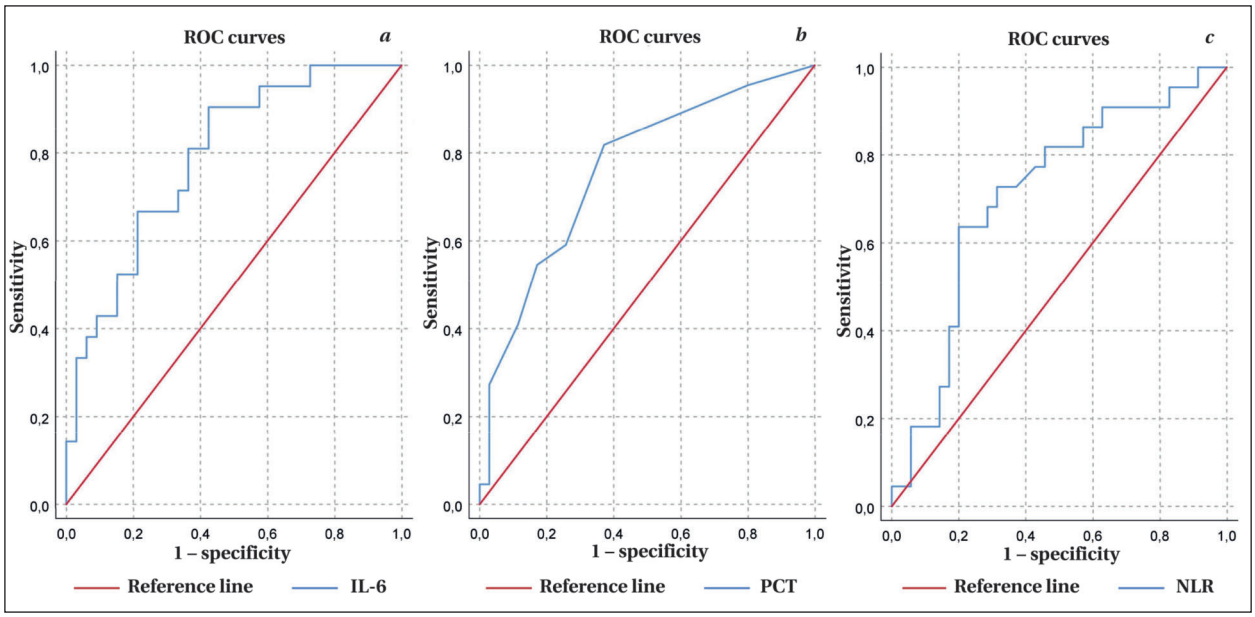

Fig. 2. Results of ROC analysis.
Note. *a* — IL-6 upon ICU admission; *b* — PCT 6 hours after ICU admission; *c* — NLR on the 3rd day after surgery.

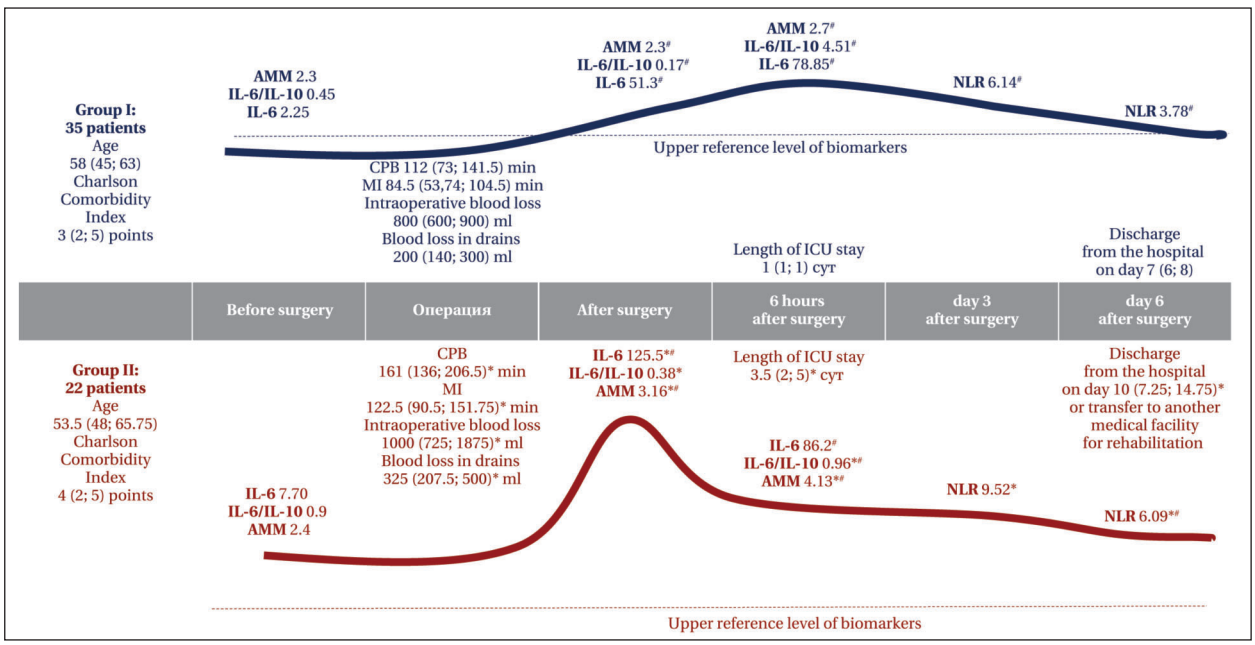

Fig. 3. Original flow chart of the perioperative course in patients without complications (group 1) and with complications (group 2).
Note. * — $P<0.005$ compared to previous measurements; # — $P<0.005$ compared to the other group.

cyte count [21]. In patients undergoing open-heart surgery, significant differences in body temperature, leukocyte count, and CRP levels were observed during the first three days, but these did not reflect the development of infection. Only after the sixth day did a renewed increase in these parameters indicate the presence of infection [22].

There were no differences in leukocyte counts between the groups studied. More informative markers included PCT, IL-6, aromatic microbial metabolites, and hematologic indices (HI).

According to other research groups, PCT thresholds vary widely depending on the presence of comorbidities, the timing of marker assessment, and other factors [23]. For example, P. Sharma and colleagues reported that on the first postoperative day, a PCT concentration > 7 ng/mL indicates a high risk of infectious complications [24].

In our study, PCT levels in the very early postoperative period (6 hours after ICU admission) showed high sensitivity (90.9%) with relatively low specificity (54.3%), making it a convenient marker for initial assessment. A PCT concentration < 0.12 ng/mL in the early postoperative period suggests an adaptive response to surgical stress and a low likelihood of complications. However, when evaluating PCT concentrations, it is important to adjust diagnostic thresholds in the presence of renal dysfunction [25].

In cases of uncertainty, additional biomarkers with high specificity can be used, such as tumor necrosis factor-alpha (TNF-α) and IL-6/IL-10 measured 6 hours after surgery. Among these, IL-6 at ICU admission showed the most optimal combination of sensitivity and specificity (> 143.35 pg/mL; 42.9% and 90.9%, respectively). The high specificity of IL-6 for early infectious complications has been confirmed in other studies [26, 27]. Elevated IL-6 levels after CPB have also been associated with hyperdynamic cardiovascular instability and metabolic disturbances [28].

Notably, the changes of IL-6 as a pro-inflammatory marker may be more clinically relevant than the absolute level above a certain threshold. IL-6 levels at ICU admission correlated with almost all biomarkers studied (PCT levels, AMM levels and their perioperative variations, NLR), as well as with CPB and MI duration, total blood loss, and ICU length of stay.

One of the circulating metabolites of the microbiota is p-hydroxyphenylacetic acid (p-HPAA). During sepsis, microbial metabolism of aromatic amino acids occurs not only in the gastrointestinal tract but also in inflammation sites. This leads to an excessive release of metabolic intermediates into the bloodstream, increasing the levels of sepsis-associated circulating metabolites, including p-HPAA [29].

In our study, p-HPAA concentrations exceeded 1.1 μM immediately after ICU admission and 6 hours later. This marker showed the highest specificity (94.3% and 94.1%, respectively) but relatively low sensitivity (27.3% and 36.4%, respectively). According to the findings of N. Beloborodova et al., healthy volunteers typically have p-HPAA concentrations below 0.5 μM, whereas patients with sepsis have significantly higher levels, averaging 2.1 μM (1.7–7.0 μM) [29].

Thus, a p-HPAA level > 1.1 μM after surgery indicates a high risk of developing inflammatory complications, even if it does not reach levels characteristic of septic patients.

In a previous study, the risk of all types of postoperative complications in cardiac surgery patients was evaluated, including the total concentration of aromatic microbial metabolites (∑AMM) six hours after surgery. The prognostic value of ∑AMM was found to be moderate, with an area under the curve (AUC) of 0.717, a threshold of 2.9 μM, a sensitivity of 81%, and a specificity of 56% [30].

In the present study, the same threshold of 2.9 μM demonstrated a lower prognostic significance (AUC of 0.705, sensitivity of 77.3% and specificity of 51.1%). However, after ICU admission, this parameter showed a higher prognostic significance with a sensitivity of 81.8% and a specificity of 60%, which is crucial for timely diagnostic and therapeutic decisions. In addition, the dynamic changes in total aromatic microbial metabolite levels before and after surgery, as well as from preoperative levels to six hours after ICU admission, showed some of the highest sensitivities (77.3% and 72.7%, respectively) and specificities (71.4% and 71.4%, respectively) among all biomarkers analyzed.

Hematological indices, calculated from routine complete blood counts available in any hospital, provide a practical tool for monitoring throughout the postoperative period, especially in the absence of more advanced laboratory diagnostics. On the one hand, HI reflect the body's response to CPB (since CPB significantly alters neutrophil and lymphocyte counts and their characteristics) [31]. On the other hand, they have already been established as predictors of adverse outcomes after various cardiac surgical procedures [32–34].

According to Y. Zhu et al., a postoperative NLR > 7.5 in patients undergoing CPB surgery is associated with higher 30-day mortality rates [35]. In our study, an NLR > 7.8 on postoperative day 3 demonstrated predictive value for the development of infectious complications with a sensitivity of 72.7% and a specificity of 68.6%. At postoperative day 6, an NLR > 5.4 showed decreased sensitivity (68.2%) but increased specificity (80%), suggesting that at postoperative day 6, NLR is more appropriately considered a marker of an active infection rather than a predictor of potential infection.

Study limitations. Limitations of this study include the lack of a priori sample size calculation, the small number of observations, and the high degree of heterogeneity in the population of cardiac surgery patients undergoing aortic reconstruction. Patient recruitment is ongoing to address these limitations.

Conclusion

Dysregulated systemic inflammatory responses immediately after aortic reconstructive surgery are detected by elevated PCT levels > 0.12 ng/mL, IL-6 levels > 143.35 pg/mL, and the presence of AMM, especially p-HPAA > 1.1 μM. NLR values > 7.8 and > 5.4 on the third and sixth postoperative day, respectively, serve as markers of a developing infection and inflammation.

Appendix

Table A1. Comprehensive list of measured biomarkers.

Name	Units of measurement
Basophils, absolute count	10^3 cells/μL
BA — Benzoic acid	μM
HVA — Homovanillic acid	μM
Leukocytes, absolute count	10^9 cells/L
Lymphocytes, absolute count	10^3 cells/μL
Monocytes, absolute count	10^3 cells/μL
Immature granulocytes, absolute count	10^9 cells/L
Neutrophils, absolute count	10^3 cells/μL
p-HBA — p-hydroxybenzoic acid	μM
p-HPLA — p-hydroxyphenyllactic acid	μM
p-HPPA — p-hydroxyphenylpropionic acid	μM
p-HPAA — p-hydroxyphenylacetic acid	μM
NLR — Neutrophil-to-lymphocyte ratio	
Platelets	10^9 cells/L
PLA — Phenyllactic acid	μM
TNF-α — Tumor necrosis factor-alpha	pg/L
PPA — Phenylpropionic acid	μM
Eosinophils, absolute count	10^3 cells/μL
TSH — Thyroid-stimulating hormone (high-sensitivity)	μIU/mL
IL-10	pg/mL
IL-1β	pg/mL
IL-6	pg/mL
IL-8	pg/mL
NLR — Neutrophil-to-lymphocyte ratio	
NSE — Neuron-specific enolase	ng/mL
NT-proBNP — N-terminal pro-brain natriuretic peptide	pg/mL
PCT — Procalcitonin	ng/mL

Table A2. Results of ROC analysis.

Parameter	Threshold Level	Sensitivity	Specificity	AUC	95% CI	*P* value
p-HPAA						
— Upon admission to ICU	>1.1	27.3	94.3	0.685	0.538–0.832	0.013
— 6 hours after admission to ICU	>1.1	36.4	94.1	0.765	0.634–0.896	<0.001
∑AMM						
— Upon admission to ICU	>2.5	81.8	60	0.710	0.576–0.845	0.002
— 6 hours after admission to ICU	>2.3	77.3	51.1	0.705	0.562–0.847	0.005
△AMM						
— Pre- and post-operation	>0.185	77.3	71.4	0.780	0.651–0.909	<0.001
— Pre-op and 6 hours post-admission	>0.354	72.7	71.4	0.713	0.565–0.861	0.005
PCT						
— 6 hours after admission to ICU	>0.1185	90.9	54.3	0.762	0.634–0.891	<0.001
IL-6						
— Upon admission to ICU	>143.35	42.9	90.9	0.789	0.669–0.909	<0.001
IL-6/IL-10						
— Upon admission to ICU	>3.865	14.3	94.1	0.671	0.531–0.818	0.017
— 6 hours after admission to ICU	—	—	—	0.665	0.494–0.835	0.058
TNF-α						
— 6 hours post-operation	>9.6	9.5	91.2	0.676	0.534–0.818	0.015
Neutrophils						
— 6th day post-operation	>5.45	95.5	37.1	0.688	0.548–0.828	0.008
Lymphocytes						
— 3rd day post-operation	<1.25	81.8	65.7	0.727	0.589–0.865	0.001
— 6th day post-operation	<1.05	36.4	85.7	0.673	0.525–0.822	0.022
NLR						
— 3rd day post-operation	>7.8350	72.7	68.6	0.710	0.571–0.850	0.003
— 6th day post-operation	>5.424	68.2	80	0.742	0.609–0.874	<0.001

Table A3. Results of correlation analysis.

Parameter 1	Parameter 2	*R*	*P* value
PCT before surgery, ng/mL	p-HPAA 6 hours after ICU admission, μM	0.360	0.006
IL-6 upon ICU admission, ng/mL	p-HPAA upon ICU admission, μM	0.404	0.002
IL-6 upon ICU admission, ng/mL	p-HPAA 6 hours after ICU admission, μM	0.423	0.002
TNF-α 6 hours after ICU admission, pg/L	p-HPAA 6 hours after ICU admission, μM	0.421	0.002
IL-6/IL-10 upon ICU admission	p-HPAA upon ICU admission, μM	0.345	0.010
IL-6/IL-10 upon ICU admission	p-HPAA 6 hours after ICU admission, μM	0.356	0.008
PCT 6 hours after ICU admission, ng/mL	∑AMM upon ICU admission, μM	0.312	0.018
PCT 6 hours after ICU admission, ng/mL	∑AMM 6 hours after ICU admission, μM	0.411	0.001
IL-6 upon ICU admission, ng/mL	∑AMM upon ICU admission, μM	0.422	0.001
IL-6 upon ICU admission, ng/mL	∑AMM 6 hours after ICU admission, μM	0.317	0.020
PCT 6 hours after ICU admission, ng/mL	△AMM (pre-surgery vs, post-surgery)	0.480	<0.001
PCT 6 hours after ICU admission, ng/mL	△AMM (pre-surgery vs, 6 hours post-surgery)	0.461	<0.001
IL-6 upon ICU admission, ng/mL	△AMM (pre-surgery vs, post-surgery)	0.364	0.007
IL-6 upon ICU admission, ng/mL	PCT 6 hours after ICU admission, ng/mL	0.543	<0.001
IL-6 upon ICU admission, ng/mL	TNF-α 6 hours after ICU admission, pg/L	0.381	0.004
IL-6/IL-10 upon ICU admission	TNF-α 6 hours after ICU admission, pg/L	0.307	0.023
△AMM (pre-surgery vs, post-surgery)	Neutrophils (absolute count), 6th day post-surgery, 10^3 cells/μL	0.280	0.035
PCT 6 hours after ICU admission, ng/mL	Neutrophils (absolute count), 6th day post-surgery, 10^3 cells/μL	0.387	0.003
p-HPAA 6 hours after ICU admission, μM	Lymphocytes (absolute count), 3rd day post-surgery, 10^3 cells/μL	–0.285	0.034
△AMM (pre-surgery vs, 6 hours post-surgery)	Lymphocytes (absolute count), 6th day post-surgery, 10^3 cells/μL	–0.362	0.006
PCT 6 hours after ICU admission, ng/mL	Lymphocytes (absolute count), 3rd day post-surgery, 10^3 cells/μL	–0.296	0.025
IL-6 upon ICU admission, ng/mL	Lymphocytes (absolute count), 6th day post-surgery, 10^3 cells/μL	–0.330	0.015
p-HPAA 6 hours after ICU admission, μM	NLR, 3rd day post-surgery	0.272	0.042
△AMM (pre-surgery vs, post-surgery)	NLR, 3rd day post-surgery	0.299	0.001
△AMM (pre-surgery vs, 6 hours post-surgery)	NLR, 3rd day post-surgery	0.424	0.001
IL-6 upon ICU admission, ng/mL	NLR, 6th day post-surgery	0.358	0.008
PCT 6 hours after ICU admission, ng/mL	NLR, 6th day post-surgery	0.368	0.005

Table A4. Results of correlation analysis of biomarkers with perioperative characteristics.

Parameter	Days in ICU	Days in the ward	Days total	Age	CCI	CPB	MI	Intra-operative blood loss	Drains	Total blood loss
Preoperative p-HPAA					0.261*					
p-HPAA upon ICU admission					0.295*					
∑AMM upon ICU admission	0.346#				0.291*	0.290*			0.291*	
∑AMM 6 hrs after ICU admission	0.398#				0.288*		0.312*	0.311*	0.362#	0.335*
△AMM (pre- and post-op)	0.550#		0.327*			0.371#	0.284*			
△AMM (pre-op and 6 hrs post-op)	0.410#		0.268*				0.292*			
PCT 6 hrs after ICU admission	0.525#		0.369#			0.385#	0.415#	0.441#	0.314*	0.478#
IL-6 upon ICU admission	0.472#		0.402#			0.376#	0.455#		0.279*	0.312*
IL-6/IL-10 upon ICU admission			0.300*				0.287*			
TNF-α 6 hrs after ICU admission			0.276*	0.348#	0.361#				0.308*	
Neutrophils 6 days post-op	0.384#		0.315*					0.261*		
Lymphocytes 3 days post-op	–0.443#		–0.342#		–0.265*	–0.356#	–0.346*			
Lymphocytes 6 days post-op	–0.291*	–0.264*	–0.370#			–0.306*	–0.360*	–0.322*	–0.274*	–0.375#
NLR 3 days post-op	0.314*			0.377#	0.317*					
NLR 6 days post-op	0.438#	0.291*	0.462#			0.352#	0.436#	0.395#	0.324*	0.426#

Note. * — $P<0.05$; # — $P<0.001$.

References

1. *Попов Д. А.* Послеоперационные инфекционные осложнения в кардиохирургии. *Анналы хирургии*. 2013; (5): 15–21. *Popov D. A.* Postoperative infectious complications in cardiac surgery. *Annals of Surgery = Annaly Khirurgii*. 2013; (5): 15–21. (in Russ.).
2. *Robich M. P., Sabik J. F. 3rd, Houghtaling P. L., Kelava M., Gordon S., Blackstone E. H., Koch C. G.* Prolonged effect of postoperative infectious complications on survival after cardiac surgery. *Ann Thorac Surg*. 2015; 99 (5): 1591–1599.
DOI: 10.1016/j.athoracsur.2014.12.037.
PMID: 25686669.
3. *Jenney A. W., Harrington G. A., Russo P. L., Spelman D. W.* Cost of surgical site infections following coronary artery bypass surgery. *ANZ J Surg*. 2001; 71 (11): 662–664.
DOI: 10.1046/j.1445-1433.2001.02225.x.
PMID: 11736828.
4. *Jonkers D., Elenbaas T., Terporten P., Nieman F., Stobberingh E.* Prevalence of 90-days postoperative wound infections after cardiac surgery. *Eur J Cardiothorac Surg*. 2003; 23 (1): 97–102.
DOI: 10.1016/s1010-7940(02)00662-0.
PMID: 12493512.
5. *Aljure O. D., Fabbro M. 2nd*. Cardiopulmonary bypass and inflammation: the hidden enemy. *J Cardiothorac Vasc Anesth*. 2019; 33 (2): 346–347.
DOI: 10.1053/j.jvca.2018.05.030.
PMID: 30057250.
6. *Paparella D., Yau T. M., Young E.* Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update. *Eur J Cardiothorac Surg*. 2002; 21 (2): 232–244.
DOI: 10.1016/s1010-7940(01)01099-5.
PMID: 11825729.
7. *Maisat W., Yuki K.* Narrative review of systemic inflammatory response mechanisms in cardiac surgery and immunomodulatory role of anesthetic agents. *Ann Card Anaesth*. 2023; 26 (2): 133–142.
DOI: 10.4103/aca.aca_147_22.
PMID: 37706376.
8. *Kraft F., Schmidt C., Van Aken H., Zarbock A.* Inflammatory response and extracorporeal circulation. *Best Pract Res Clin Anaesthesiol*. 2015; 29 (2): 113–123.
DOI: 10.1016/j.bpa.2015.03.001.
PMID: 26060024.
9. *Jayakumar S., Khoynezhad A., Jahangiri M.* Surgical site infections in cardiac surgery. *Crit Care Clin*. 2020; 36 (4): 581–592.
DOI: 10.1016/j.ccc.2020.06.006.
PMID: 32892815.
10. *Michalopoulos A., Geroulanos S., Rosmarakis E. S., Falagas M. E.* Frequency, characteristics, and predictors of microbiologically documented nosocomial infections after cardiac surgery. *Eur J Cardiothorac Surg*. 2006; 29 (4): 456–460.
DOI: 10.1016/j.ejcts.2005.12.035.
PMID: 16481186.
11. *Kollef M. H., Sharpless L., Vlasnik J., Pasque C., Murphy D., Fraser V. J.* The impact of nosocomial infections on patient outcomes following cardiac surgery. *Chest*. 1997; 112 (3): 666–675.
DOI: 10.1378/chest.112.3.666. PMID: 9315799.
12. *Damavandi D. S., Javan M., Moshashaei H., Forootan M., Darvishi M.* Microbial contamination after cardiac surgery in a hospital cardiac surgery ward. *J Med Life*. 2020; 13 (3): 342–348.
DOI: 10.25122/jml-2019-0071.
PMID: 33072206.
13. *Orita H., Shimanuki T., Fukasawa M., Inui K., Goto S., Washio M., Horikawa H.* A clinical study of postoperative infections following open-heart surgery: occurrence and microbiological findings in 782 cases. *Surg Today*. 1992; 22 (3): 207–212.
DOI: 10.1007/BF00308824. PMID: 1392323.
14. *Paternoster G., Guarracino F.* Sepsis after cardiac surgery: from pathophysiology to management. *J Cardiothorac Vasc Anesth*. 2016; 30 (3): 773–780.
DOI: 10.1053/j.jvca.2015.11.009.
PMID: 26947713.
15. *Гринь О. О., Бабаев М. А., Грекова М. С., Котельникова Е. О., Исалова К. М., Еременко А. А.* Предиктивная значимость гематологических индексов при реконструктивных операциях на аорте: проспективное наблюдательное исследование. *Вестник интенсивной терапии им. А. И. Салтанова*. 2024; 1: 82–93. *Grin O. O., Babaev M. A., Grekova M. S., Kotelnikova E. O., Isalova K. M., Eremenko A. A.* Predictive significance of hematological indices in aortic reconstructive surgery: a prospective observational study. *Ann Crit Care = Vestnik Intensivnoy Terapii im AI Saltanova*. 2024; 1: 82–93. (in Russ.).
DOI: 10.21320/1818-474X-2024-1-82-9.
16. Нозокомиальная пневмония у взрослых: Российские национальные рекомендации. Под ред. акад. РАН Б. Р. Гельфанда; отв. ред. к. м. н., доцент Д. Н. Проценко, к. м. н., доцент Б. З. Белоцерковский. — 2-е изд., перераб. и доп. — М.: ООО «Издательство «Медицинское информационное агентство»; 2016: 176. Nosocomial pneumonia in adults: Russian national recommendations. Ed. acad. RAS B. R. Gelfand; ed. Candidate of Medical Sciences, Associate Professor D. N. Protsenko, Candidate of Medical Sciences, Associate Professor B. Z. Belotserkovsky. — 2nd ed., revised and exp. M.: Publishing House «Medical Information Agency» LLC; 2016: 176. ISBN 978-5-9986-0284-9.

17. *Smid J., Scherner M., Wolfram O., Groscheck T., Wippermann J., Braun-Dullaeus R. C.* Cardiogenic causes of fever. *Dtsch Arztebl Int.* 2018; 115 (12): 193–199.
DOI: 10.3238/arztebl.2018.0193.
PMID: 29642989.
18. *Garibaldi R. A., Brodine S., Matsumiya S., Coleman M.* Evidence for the non-infectious etiology of early postoperative fever. *Infect Control.* 1985; 6 (7): 273–277.
DOI: 10.1017/s0195941700061749.
PMID: 3847403.
19. *Livelli F. D. Jr., Johnson R. A., McEnany M.T., Sherman E., Newell J., Block P. C., DeSanctis R. W.* Unexplained in-hospital fever following cardiac surgery. Natural history, relationship to postpericardiotomy syndrome, and a prospective study of therapy with indomethacin versus placebo. *Circulation.* 1978; 57 (5): 968–975.
DOI: 10.1161/01.cir.57.5.968. PMID: 346257.
20. *Rhee C., Sax P. E.* Evaluation of fever and infections in cardiac surgery patients. 2015; 19 (2): 143–153.
DOI: 10.1177/1089253214538524.
PMID: 24958717.
21. *Bateman R. M., Sharpe M. D., Jagger J. E., Ellis C. G., Solle-Volan J., Lopez-Rodrigez M., Herrera-Ramos E., et al.* 36th International Symposium on Intensive Care and Emergency Medicine: Brussels, Belgium. 15–18 March 2016. *Crit Care.* 2016; 20 (Suppl 2): 94.
DOI: 10.1186/s13054-016-1208-6.
PMID: 27885969.
22. *Verkkala K., Valtonen V., Järvinen A., Tolppanen E. M.* Fever, leucocytosis and C-reactive protein after open-heart surgery and their value in the diagnosis of postoperative infections. *Thorac Cardiovasc Surg.* 1987; 35 (2): 78–82.
DOI: 10.1055/s-2007-1020201. PMID: 2440139.
23. *Smith S. E., Muir J., Kalabalik-Hoganson J.* Procalcitonin in special patient populations: guidance for antimicrobial therapy. *Am J Health Syst Pharm.* 2020; 77 (10): 745–758.
DOI: 10.1093/ajhp/zxaa089. PMID: 32340027.
24. *Sharma P., Patel K., Baria K., Lakhia K., Malhotra A., Shah K., Patel S.* Procalcitonin level for prediction of postoperative infection in cardiac surgery. *Asian Cardiovasc Thorac Ann.* 2016; 24 (4): 344–349.
DOI: 10.1177/0218492316640953.
PMID: 27002098.
25. *de la Varga-Martínez O., Martín-Fernández M., Heredia-Rodríguez M., Ceballos F., Cubero-Gallego H., Priede-Vimbela J. M., Bardají-Carrillo M., et al.* Influence of renal dysfunction on the differential behaviour of procalcitonin for the diagnosis of postoperative infection in cardiac surgery. *J Clin Med.* 2022; 11 (24): 7274.
DOI: 10.3390/jcm11247274. PMID: 36555891.
26. *Xie M., Chen Y. T., Zhang H., Zhang H. T., Pan K., Chen X. F, Xie X. L.* Diagnostic value of procalcitonin and interleukin-6 on early postoperative pneumonia after adult cardiac surgery: a prospective observational study. *Heart Surg Forum.* 2022; 25 (1): E020–E029.
DOI: 10.1532/hsf.4297. PMID: 35238309.
27. *Sander M., von Heymann C., von Dossow V., Spaethe C., Konertz W. F, Jain U., Spies C. D.* Increased interleukin-6 after cardiac surgery predicts infection. *Anesth Analg.* 2006; 102 (6): 1623–1629.
DOI: 10.1213/01.ane.0000215998.21739.48.
PMID: 16717298.
28. *Cremer J., Martin M., Redl H., Bahrami S., Abraham C., Graeter T., Haverich A., et al.* Systemic inflammatory response syndrome after cardiac operations. *Ann Thorac Surg.* 1996; 61 (6): 1714–1720.
DOI: 10.1016/0003-4975 (96)00055-0.
PMID: 8651772.
29. *Черневская Е. А., Гецина М. Л., Черпаков Р. А., Сорокина Е. А., Шабанов А. К., Мороз В. В., Белобородова Н. В.* Сепсис-ассоциированные метаболиты и их биотрансформация кишечной микробиотой. *Общая реаниматология*. 2023; 19 (6): 4–12. *Chernevskaya E. A., Getsina M. L., Cherpakov R. A., Sorokina E. A., Shabanov A. K., Moroz V. V., Beloborodova N. V.* Sepsis-associated metabolites and their biotransformation by intestinal microbiota. *General Reanimatology = Obshchaya Reanimatologiya.* 2023; 19 (6): 4–12. (in Russ.&Eng.).
DOI: 10.15360/1813-9779-2023-6-4-12.
30. *Beloborodova N., Pautova A., Grekova M., Yadgarov M., Grin O., Eremenko A., Babaev M.* Microbiota metabolism failure as a risk factor for postoperative complications after aortic prosthetics. *Biomedicines.* 2023; 11 (5): 1335.
DOI: 10.3390/biomedicines11051335.
PMID: 37239006.
31. *Nguyen M., Stiel L., Guilloteau A., Bahr P. A., Masson D., Thomas C., Blot M., et al.* Leukocyte cell population data in patients with cardiac surgery and cardiopulmonary bypass: a potential readily available tool to monitor immunity. *Front Immunol.* 2023; 13: 1101937.
DOI: 10.3389/fimmu.2022.1101937.
PMID: 36741408.
32. *Gibson P. H., Croal B. L., Cuthbertson B. H., Small G. R., Ifezulike A. I., Gibson G., Jeffrey R. R., et al.* Preoperative neutrophil-lymphocyte ratio and outcome from coronary artery bypass grafting. *Am Heart J.* 2007; 154 (5): 995–1002.
DOI: 10.1016/j.ahj.2007.06.043.
PMID: 17967611.

33. *King A. H., Schmaier A. H., Harth K. C., Kumins N. H., Wong V. L., Zidar D. A., Kashyap V. S., et al.* Elevated neutrophil-lymphocyte ratio predicts mortality following elective endovascular aneurysm repair. *J Vasc Surg.* 2020; 72 (1): 129–137.
DOI: 10.1016/j.jvs.2019.10.058.
PMID: 32037083.
34. *Khalil C., Pham M., Sawant A. C., Sinibaldi E., Bhardwaj A., Ramanan T., Qureshi R., et al.* Neutrophil-to-lymphocyte ratio predicts heart failure readmissions and outcomes in patients undergoing transcatheter aortic valve replacement. *Indian Heart J.* 2018; 70 Suppl 3 (Suppl 3): S313-S318.
DOI: 10.1016/j.ihj.2018.08.002.
PMID: 30595282.
35. *Zhu Y., Peng W., Zhen S., Jiang X.* Postoperative neutrophil-to-lymphocyte ratio is associated with mortality in adult patients after cardiopulmonary bypass surgery: a cohort study. *Med Sci Monit.* 2021; 27: e932954.
DOI: 10.12659/MSM.932954. PMID: 34565791.

Received 25.06.2024
Accepted 26.11.2024

https://doi.org/10.15360/1813-9779-2025-1-2517

Correlation of Immune Parameters in Breast Cancer Patients Undergoing General Anesthesia: Post-hoc Analysis of the TeMP Study

Kristina K. Kadantseva[1,2]*, Valery V. Subbotin[2], Mikhail Y. Yadgarov[1]*, Elizaveta M. Korolenok[1], Levan B. Berikashvili[1], Roman A. Akchulpanov[2], Nikolay S. Karnaukhov[2], Ksenia S. Korchagina[2], Polina I. Kukina[3], Oksana A. Svitich[3], Artem N. Kuzovlev[1], Anna S. Barmina[4], Valery V. Likhvantsev[1,5]

[1] Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[2] A. S. Loginov Moscow Clinical Research Center, Moscow Department of Health, 86 Enthusiasts Highway, 111123 Moscow, Russia
[3] I. I. Mechnikov Research Institute of Vaccines and Serums, 5a Maly Kazenny per., 105064 Moscow, Russia
[4] N. I. Pirogov Russian National Medical Research University, Ministry of Health of Russia, 1 Ostrovityanov Str., 117513 Moscow, Russia
[5] I. M. Sechenov First Moscow State Medical University, Ministry of Health of Russia, 8 Trubetskaya Str., Bldg. 2, 119991 Moscow, Russia

For citation: *Kristina K. Kadantseva, Valery V. Subbotin, Mikhail Y. Yadgarov, Elizaveta M. Korolenok, Levan B. Berikashvili, Roman A. Akchulpanov, Nikolay S. Karnaukhov, Ksenia S. Korchagina, Polina I. Kukina, Oksana A. Svitich, Artem N. Kuzovlev, Anna S. Barmina, Valery V. Likhvantsev.* Correlation of Immune Parameters in Breast Cancer Patients Undergoing General Anesthesia: Post-hoc Analysis of the TeMP Study. *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 15–27. https://doi.org/10.15360/1813-9779-2025-1-2517 [In Russ. and Engl.]

***Correspondence to:** Kristina K. Kadantseva, kristina161093@gmail.com; Mikhail Y. Yadgarov, mikhail.yadgarov@mail.ru

Summary

Aim: to study the correlation of immune parameters in breast cancer patients undergoing general anesthesia and to evaluate the 1-year overall and recurrence-free survival after surgery depending on general anesthesia technique.

Materials and Methods. A post hoc analysis of data from a double-blind, randomized, controlled clinical trial involving 98 patients with operable breast cancer was performed. Patients were divided into two groups: 48 received inhalational anesthesia (IA) and 50 received total intravenous anesthesia (TIVA). Immune parameters (CRP, IgA, IgM, IgG, C3, C4, MMP-9, neutrophil and lymphocyte counts, etc.) were assessed before induction of anesthesia, 1 hour postoperatively, and 24 hours postoperatively. Spearman correlation coefficients and heat maps were used for analysis.

Results. In the IA group, significant uniform increases were observed in all immunoglobulin types at 1 and 24 hours postoperatively (all $P<0.001$; for IgA-IgG, $R=0.928$; for IgA-IgM, $R=0.837$; for IgG-IgM, $R=0.815$). A positive correlation was found between complement components (C3, C4) and immunoglobulins ($P=0.011$ — 0.023; $R=0.313$–0.363). In the TIVA group, changes were variable: immunoglobulin levels increased at 1 hour ($P<0.001$) but decreased at 24 hours ($P<0.001$). A strong positive correlation was identified between cytotoxic T cells and NK cells ($P<0.001$; $R=0.722$). Neutrophil count showed no significant correlation with cytotoxic T or NK cells. One year after surgery, both groups demonstrated 100% overall and recurrence-free survival.

Conclusion. IA was associated with synchronized changes in humoral immunity components, whereas TIVA resulted in variable immune responses, suggesting potential differences in IA and TIVA effects on the immune system. However, no impact of anesthesia technique on overall or recurrence-free survival was observed. More research is needed to better understand how different anesthetics affect immune function and the potential impact of anesthesia technique on long-term cancer outcomes.

Keywords: immune parameters; inhalational anesthesia; total intravenous anesthesia; breast cancer; humoral immunity;, cellular immunity; overall survival; recurrence-free survival

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

Funding. The study was supported by a grant from the Russian Science Foundation, No. 23-25-00219 (https://rscf.ru/project/23-25-00219).

Information about the authors:
Kristina K. Kadantseva: ORCID 0000-0002-6593-8580
Valery V. Subbotin: ORCID 0000-0002-0921-7199
Mikhail Ya. Yadgarov: ORCID 0000-0003-3792-1682
Elizaveta M. Korolenok: ORCID 0009-0007-5731-9273
Levan B. Berikashvili: ORCID 0000-0001-9267-3664
Roman A. Akchulpanov: ORCID 0000-0001-9983-0925
Nikolay S. Karnaukhov: ORCID 0000-0003-0889-2720
Ksenia S. Korchagina: ORCID 0000-0003-3181-1191
Polina I. Kukina: ORCID 0000-0002-0054-6754

Oksana A. Svitich: ORCID 0000-0003-1757-8389
Artem N. Kuzovlev: ORCID 0000-0002-5930-0118
Anna S. Barmina: ORCID 0009-0005-4429-4244
Valery V. Likhvantsev: ORCID 0000-0002-5442-6950

Introduction

Surgery is the primary treatment strategy for most patients with solid tumors. However, early tumor recurrence occurs in approximately one third of patients after surgery [1]. The stress response to surgery, involving activation of pro-inflammatory pathways and stimulation of angiogenesis, promotes cancer cell growth and metastasis, potentially leading to locoregional recurrence or distant metastasis [1]. While the negative impact of surgical stress on metastasis and oncologic outcomes is well understood, the effects of various anesthetic agents on these processes are less clear [2, 3]. Anesthetics can modulate the immune status of patients, significantly influencing postoperative complications such as infection and delayed tumor recurrence [2, 4]. Inhalation anesthetics have been shown to affect hypoxia-inducible factor-1 (HIF-1) and insulin-like growth factor-1 (IGF-1), with potential mechanisms involving downregulation of genes associated with angiogenesis, proliferation, and cell metabolism, leading to unfavorable oncologic prognosis [5]. Consequently, more randomized controlled trials are investigating the impact of the two most commonly used types of anesthesia (inhalation and total intravenous) on long-term oncologic outcomes and the mechanisms of immune suppression [6–9].

Current evidence suggests that inhaled anesthetics may affect both adaptive immune components (including T lymphocytes, CD4+ and CD8+ cells, and B lymphocytes) and innate immune elements (such as phagocytes including macrophages and neutrophils, and NK cells). Inhalation anesthesia (IA) and total intravenous anesthesia (TIVA) are thought to have distinct effects on various aspects of the immune system. To accurately determine how anesthesia influences immune responses, a comprehensive array of immune markers must be examined [10, 11].

The aim of this study was to investigate the relationship between immune parameters in breast cancer patients undergoing anesthesia. In addition, the one-year overall and recurrence-free survival rates following breast cancer surgery were to be assessed, taking into account the type of anesthesia used.

Materials and Methods

Study design. A post hoc analysis of a prospective, randomized, controlled, double-blind clinical trial was performed. The protocol was approved by the local ethics committee (No. 2/2021) and registered on clinicaltrials.gov (NCT04800393). Detailed descriptions of the study population, anesthesia methods, blood sampling, and data collection are available in the original publication [12].

Anesthesia methods. Patients enrolled in the study were not premedicated. Intraoperative monitoring included electrocardiography (ECG), pulse oximetry, and noninvasive arterial blood pressure measurement. Anesthesia induction in both the total intravenous anesthesia (TIVA) and inhalation anesthesia (IA) groups was performed with propofol (1.5–2.2 mg/kg), fentanyl (3–5 mcg/kg), and a neuromuscular blocking agent (rocuronium bromide or cisatracurium). Neuromuscular blockade was maintained at a train-of-four (TOF) ratio of 10–0%. Tracheal intubation was performed with an appropriately sized tube.

After induction and tracheal intubation, patients in both groups received pressure-controlled, volume-guaranteed lung ventilation (LV) using General Electric Avance CS2 or General Electric Medical Systems (USA) anesthesia machines. Ventilation parameters included: fraction of inspired oxygen (FiO_2) 35–40%, tidal volume (V_t) 6–8 mL/kg, positive end-expiratory pressure (PEEP) 5 cm H_2O, inspiratory-to-expiratory ratio (I:E) 1:2, and a respiratory rate sufficient to maintain normocapnia (35–45 mmHg).

In the TIVA group, anesthesia was maintained with propofol at a continuous infusion rate of 0.1–0.2 mg/kg/min delivered via a Braun Infusomat Space infusion pump using the Schneider model. In the IA group, anesthesia was maintained with sevoflurane administered endotracheally at 1 minimum alveolar concentration (MAC). The gas mixture consisted of 35–40% oxygen and air without nitrous oxide (N_2O). Gas flow rates were adjusted as needed to optimize oxygenation and ventilation parameters. Mean arterial pressure was maintained above 60 mmHg throughout the procedure.

After skin closure, patients were transferred to Pressure Support Ventilation-Pro (PSV-Pro) mode with the following settings: flow trigger sensitivity of 0.2 L/min, pressure support tailored to achieve a tidal volume of 6–8 mL/kg, peak airway pressure limit of 35 cm H_2O, and positive end-expiratory pressure (PEEP) of 5–8 cm H_2O. Normocapnia was maintained during this period. Tracheal extubation was performed when the TOF ratio reached $\geqslant 0.95$.

Patients were transferred to the recovery room when a modified Aldrete score of 9 or greater was achieved.

Postoperative analgesia was administered according to local standard practice with the goal of maintaining a Visual Analog Scale (VAS) pain score below 3.

Assessment of immunologic markers. Immune cell populations were characterized by flow cytometry using specific CD markers on a BD FACSCanto II

platform (Becton Dickinson Biosciences, USA). Cell populations were identified based on forward and side scatter light parameters combined with fluorescence emission profiles. Gating strategies allowed precise delineation of immune subsets including T cells (CD3+), T helper cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), B cells (CD19+CD3-), and natural killer (NK) cells (CD3-CD16+CD56+) based on specific morphological and expression (fluorescent) profiles.

Serum levels of inflammatory and immune markers, including C-reactive protein (CRP), IgA, IgM, IgG, complement components C3 and C4, were quantified by nephelometry using a BN ProSpec laser nephelometer (Siemens Healthcare Diagnostics Products GmbH). Matrix metalloproteinase-9 (MMP-9) levels were measured by enzyme-linked immunosorbent assay (ELISA) using the Human MMP-9 Quantikine ELISA Kit (Cloud-Clone Corp.).

Endpoints. The study evaluated the correlation and dynamic changes of the following immunological parameters: C-reactive protein (CRP), IgA, IgM, IgG, complement components C3 and C4, matrix metalloproteinase-9 (MMP-9), neutrophil and lymphocyte counts, neutrophil-to-lymphocyte ratio (NLR), the proportion of T cells (CD3+), T helper cells (CD3+CD4+) and cytotoxic T cells (CD3+CD8+), the immunoregulatory index (the ratio of T helper cells to cytotoxic T cells, CD3+CD4+/CD3+CD8+), the proportions of B cells (CD19+CD3-) and natural killer (NK) cells (CD3-CD16+), and the total proportions of T, B, and NK cells.

All parameters were measured at three time points: before induction of anesthesia, 1 hour after surgery, and 24 hours after surgery.

In addition, long-term patient survival outcomes, including recurrence-free survival (RFS) and overall survival (OS), were assessed one year after randomization. Data were collected through telephone interviews and review of electronic medical records, including comprehensive instrumental and laboratory findings. Overall survival (OS) was defined as the time from enrollment to death from any cause, while recurrence-free survival (RFS) was defined as the time from enrollment to either disease recurrence or death.

Statistical Analysis. All statistical calculations were performed using IBM SPSS Statistics for Windows, version 27.0 (Armonk, NY: IBM Corp.). Quantitative variables with a normal distribution were presented as means ± standard deviation *(SD)*, while variables that did not meet the assumption of normality were reported as medians with interquartile ranges *(IQR)*. Normality was assessed using the Shapiro–Wilk test and histogram analysis.

Binary variables were analyzed using the two-tailed χ^2 test or Fisher's exact test, with the Fisher–Freeman–Halton extension applied when appropriate. Independent groups of quantitative variables were compared using the Mann–Whitney *U* test. For paired samples, analysis was conducted using the Friedman test, Dunn's post hoc test, or the Wilcoxon signed-rank test.

Correlation analysis was performed to assess the relationship between each pair of parameters by calculating Spearman's rank correlation coefficient with a 95% confidence interval *(CI)*. Correlations were evaluated for baseline parameter values across patients, within groups, and for relative changes in parameters at 1 hour and 24 hours. The strength of the correlation was interpreted according to the following scale [13]:

- $0 < |R| < 0.2$: very weak
- $0.2 \leqslant |R| < 0.4$: weak
- $0.4 \leqslant |R| < 0.6$: moderate
- $0.6 \leqslant |R| < 0.8$: strong
- $0.8 \leqslant |R| < 1$: very strong

Heatmaps were generated using Python 3.11 with the numpy (2.0.0), pandas (2.2.2), seaborn (0.13.2), and matplotlib (3.9.1) libraries.

Two-tailed tests were applied with a significance level of $P<0.05$.

Results

Patients. Between 2022 and 2023, a total of 324 patients were screened for eligibility based on the inclusion and exclusion criteria at the A. S. Loginov Moscow Clinical Scientific Center. Of these, 278 patients met the inclusion criteria, while 180 were excluded: 158 because of a history of autoimmune disease and 22 because of malignancy at another site. As a result, 98 patients were enrolled in the study, of whom 48 were assigned to the IA group and 50 to the TIVA group (Fig. 1).

Baseline demographic characteristics, clinicopathologic parameters, type of surgery, and duration of anesthesia were comparable between groups (Table 1). The median age was 62 years (interquartile range [IQR], 55–68). Radical mastectomy was performed in most patients (*N*=69, 70%). Length of stay in the post-anesthesia care unit (PACU) did

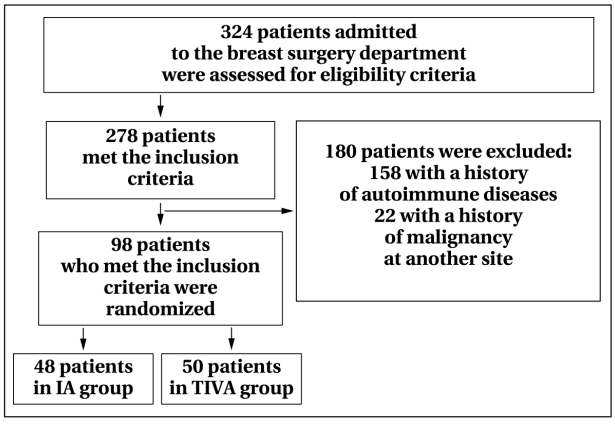

Fig. 1. Patient selection scheme for the study.
Note. For Fig. 1, 3 and Tables 1–3: TIVA — total intravenous anesthesia; IA — inhalation anesthesia.

Table 1. Main patient characteristics.

Parameter	Values in groups		*P*
	IA, *N*=48	TIVA, *N*=50	
Age, years; *N, Me (IQR)*	48, 62.5 (56–68)	50, 61 (54–68)	0.545[1]
BMI, kg/m²; *N, Me (IQR)*	46, 29 (23.8–32.0)	50, 27.6 (23.4–31.2)	0.687[1]
Comorbidities, *N* (%)			
History of COVID-19	29 (60)	27 (54)	0.521[2]
Uncontrolled diabetes mellitus	3 (6.3)	0 (0)	0.114[3]
Chronic obstructive pulmonary disease	1 (2.1)	1 (2)	0.999[3]
Cerebrovascular disease	1 (2.1)	0 (0)	0.490[3]
Peripheral arterial disease	1 (2.1)	0 (0)	0.490[3]
Diabetes mellitus	5 (10.4)	2 (4)	0.264[3]
Arterial hypertension	30 (63)	28 (56)	0.513[2]
Chronic kidney disease	0 (0)	1 (2)	0.999[3]
Coronary heart disease	3 (6.3)	4 (8)	0.999[3]
Atrial fibrillation	2 (4.2)	0 (0)	0.237[3]
Rhythm disturbances	3 (6.3)	1 (2)	0.357[3]
Heart failure	6 (12.5)	2 (4)	0.155[3]
Liver failure	0 (0)	0 (0)	NA
Dementia	0 (0)	0 (0)	NA
Rheumatic conditions	0 (0)	0 (0)	NA
Peptic ulcer disease	0 (0)	1 (2)	0.999[3]
Hemiplegia	0 (0)	0 (0)	NA
Leukemia	0 (0)	0 (0)	NA
Lymphoma	0 (0)	0 (0)	NA
AIDS/hepatitis	0 (0)	0 (0)	NA
Clinical and morphological characteristics, *N* (%) or Me (IQR)			
Tumor localization (right)	23 (48)	20 (40)	0.430[2]
TNM			
Tis	1 (2.2)	0.579[4]	0.579[4]
T1	34 (71)	31 (62)	
T2	13 (27)	17 (34)	
N0	48 (100)	NA	NA
M0	48 (100)	NA	NA
Tumor cell differentiation (G)			
G1	8 (17)	0.945[2]	0.945[2]
G2	25 (54)	26 (52)	
G3	13 (28)	14 (28)	
Stage			
0	1 (2.2)	0.204[3]	0.204[3]
IA	34 (71)	28 (56)	
IIA	13 (27)	20 (40)	
Molecular subtype			
Data not available	2 (4.2)	0.598[4]	0.598[4]
Luminal A	21 (44)	17 (34)	
Luminal B	23 (48)	28 (56)	
Triple negative	2 (4.2)	4 (8)	
Her2neu+	2 (4.2)	2 (4)	0.999[3]
TILs, %	37.3 (2–5)	38.3 (2–5)	0.961[1]
Perioperative characteristics, *N* (%) or *Me (IQR)*			
Type of surgery			
Partial resection	18 (37.5)	11 (22)	0.093[2]
Mastectomy	30 (62.5)	39 (78)	
Duration of anesthesia, minutes	87.5 (75.0–102.5)	90.0 (70.0–105.0)	0.839[1]
Duration of surgery, minutes	70.0 (60.0–90.0)	70.0 (60.0–90.0)	0.937[1]
Anesthetic injection time, min	85 (75–100)	90 (70–105)	0.908[1]
Intraoperative fentanyl dose, mg	0.3 (0.3–0.4)	0.4 (0.3–0.5)	0.534[1]
Intraoperative propofol dose, mg	1.5–2.2 mg/kg — induction of anesthesia. No — maintenance of anesthesia	800 (600–1000)	NA
Intraoperative sevoflurane dose for maintenance of anesthesia (MAC)	0.9 (0.8–1.0)	No	NA

Note. [1] — Mann–Whitney test; [2] — Chi-squared test; [3] — Fisher's exact test; [4] — Fisher–Freeman–Halton test. NA — not applicable; *Me* — median value; *IQR* — interquartile range; *N* — number of patients with the specified parameter.

not exceed 24 hours. The median length of hospital stay was 3 days (IQR, 3–5) in the IA group and 4 days (IQR, 3–5) in the TVA group (*P*=0.131).

Comparison of baseline immunologic blood markers. Statistically significant differences between the groups were found for the concentrations of

C-reactive protein, IgA and IgG, and the proportions of T and B lymphocytes and NK cells (Table 2).

Analysis of correlations of baseline immunologic parameters showed that all immunoglobulins were positively correlated with each other with moderate to strong correlation strength (R=0.515 to 0.783; all P<0.001) (Fig. 2). A weak positive correlation was observed between components of the complement

Table 2, Serum biomarkers of patients in the IA and TIVA groups, *Me [Q1; Q3]*.

Laboratory parameter		Values in groups		P^1
		IA, N=48	TIVA, N=50	
C-reactive protein, mg/L	Before surgery	**0.7 [0.29; 2.21]**	**1.49 [0.77; 3.91]**	**0.023**
	1 hour after surgery	**0.77 [0.31; 2.29]**	**1.45 [0.79; 4.01]**	**0.038**
	% change from baseline before 1 h	–3.19 [–11.29; 1.66]	–0.44 [–6.22; 6.14]	0.112
	24 h after surgery	5.84 [2.45; 10.75]	5.54 [2.87; 11.78]	0.848
	% of change from baseline to 24 h	**466.38 [191.73; 1015.35]**	**260.56 [73.29; 631.25]**	**0.044**
IgA, g/L	Before surgery	**2.33 [1.56; 3.06]**	**1.09 [0.47; 2.25]**	**<0.001**
	1 hour after surgery	**2.27 [1.55; 2.98]**	**1.42 [0.81; 2.45]**	**<0.001**
	% change from baseline before 1 h	**–3.01 [–9.34; 2.73]**	**6.22 [–8.09; 82.75]**	**0.011**
	24 h after surgery	**2.23 [1.67; 2.75]**	**1.11 [0.46; 1.99]**	**<0.001**
	% of change from baseline to 24 h	–5.64 [–15.32; 3.63]	–5.84 [–26.4; 6.48]	0.629
IgM, g/L	Before surgery	0.82 [0.51; 1.23]	0.74 [0.33; 1.11]	0.058
	1 hour after surgery	0.84 [0.61; 1.17]	0.72 [0.39; 1.22]	0.207
	% change from baseline before 1 h	**–4.55 [–13.07; 0.88]**	**9.3 [–12.04; 70.95]**	**0.031**
	24 h after surgery	**0.81 [0.58; 1.17]**	**0.57 [0.35; 0.89]**	**0.005**
	% of change from baseline to 24 h	–3.4 [–15.4; 10.84]	–9.63 [–35.68; 17.99]	0.298
IgG, g/L	Before surgery	**10.09 [8.22; 12.8]**	**5.76 [2.52; 10.6]**	**<0.001**
	1 hour after surgery	**10.05 [8.15; 11.8]**	**7.98 [3.95; 10.23]**	**<0.001**
	% change from baseline before 1 h	**–4.77 [–11.9; 1.49]**	**4.3 [–7.21; 85.7]**	**0.013**
	24 h after surgery	**9.76 [8.26; 11.2]**	**5.64 [2.11; 9.96]**	**<0.001**
	% of change from baseline to 24 h	–7.85 [–15.5; –0.76]	–8.53 [–32.06; 3.79]	0.589
C3, g/L	Before surgery	1.25 [1.08; 1.36]	1.3 [1.11; 1.49]	0.161
	1 hour after surgery	1.2 [1.03; 1.35]	1.34 [1.12; 1.48]	0.078
	% change from baseline before 1 h	–4.88 [–7.73; –0.2]	–2.41 [–8.76; 2.22]	0.350
	24 h after surgery	1.23 [1.02; 1.39]	1.31 [1.17; 1.48]	0.086
	% of change from baseline to 24 h	–2.52 [–7.65; 2.41]	0.27 [–4.5; 5.02]	0.111
C4, g/L	Before surgery	0.31 [0.25; 0.35]	0.31 [0.24; 0.36]	0.752
	1 hour after surgery	0.3 [0.23; 0.33]	0.31 [0.23; 0.35]	0.709
	% change from baseline before 1 h	–4.73 [–8.59; 0.6]	–1.86 [–7.64; 2.17]	0.346
	24 h after surgery	0.3 [0.26; 0.34]	0.31 [0.24; 0.39]	0.621
	% of change from baseline to 24 h	–2.33 [–9.56; 5.5]	1.22 [–5.94; 10.86]	0.105
MMP-9, ng/mL	Before surgery	29, 19.71 [13.71; 20]	11, 17.35 [13.38; 19.3]	0.254
	24 h after surgery	29, 20 [15.93; 20]	11, 17.39 [14.74; 20]	0.492
	% of change from baseline to 24 h	29, 0 [0; 22.3]	11, 0.2 [–1.69; 16.24]	0.905
Neutrophil count, 10^9/L	Before surgery	47, 3.7 [3.1; 4.6]	3.3 [2.5; 4]	0.086
	1 hour after surgery	47, 5.6 [4; 7]	49, 5.4 [4.2; 7.35]	0.866
	% change from baseline before 1 h	46, 60.09 [8.53; 94.55]	49, 66.67 [30.21; 131.74]	0.295
	24 h after surgery	45, 6.8 [5.2; 9.1]	49, 5.8 [4.55; 8.6]	0.217
	% of change from baseline to 24 h	44, 80.66 [36; 151.56]	49, 80 [37.52; 172.25]	0.681
Neutrophil percentage, %	Before surgery	47, 57.4 [48.4; 63.9]	55.45 [51.38; 62]	0.865
	1 hour after surgery	47, 77.9 [62.7; 87.4]	49, 78.5 [66.65; 83.75]	0.789
	% change from baseline before 1 h	47, 37.09 [10.56; 54.28]	33.13 [11.61; 53.35]	0.854
	24 h after surgery	45, 69.5 [64.35; 74.75]	49, 67.6 [63.05; 73.35]	0.440
	% of change from baseline to 24 h	47, 22.67 [6.26; 41.23]	16.85 [4.97; 38.58]	0.831
Lymphocyte count, 10^9/L	Before surgery	47, 2.06 [1.7; 2.6]	2.1 [1.5; 2.36]	0.237
	1 hour after surgery	47, 1.2 [0.7; 1.7]	1.3 [0.98; 1.62]	0.558
	% change from baseline before 1 h	46, –47.22 [–62.33; –10.48]	–36.55 [–50; –7.24]	0.055
	24 h after surgery	45, 2 [1.75; 2.45]	49, 2 [1.55; 2.5]	0.604
	% of change from baseline to 24 h	44, –7.42 [–16.79; 13.13]	49, 6.67 [–13.96; 19.7]	0.193
Lymphocyte percentage, %	Before surgery	47, 33.3 [26.5; 39]	33.75 [28.25; 38.28]	0.894
	1 hour after surgery	47, 17.2 [10.2; 26]	16.4 [11.88; 25.53]	0.928
	% change from baseline before 1 h	47, –58.61 [–65.37; –23.1]	–49.39 [–62.6; –25.05]	0.593
	24 h after surgery	45, 21.5 [15.6; 25.85]	49, 23 [18.6; 27]	0.269
	% of change from baseline to 24 h	47, –34.36 [–59.79; –21.92]	–29.41 [–47.22; –15.24]	0.165
Neutrophil-lymphocyte ratio (NLR)	Before surgery	47, 1.7 [1.24; 2.43]	1.65 [1.34; 2.18]	0.767
	1 hour after surgery	47, 4.54 [2.4; 9]	49, 4.42 [2.5; 6.78]	0.519
	% change from baseline before 1 h	47, 246.94 [36.4; 343.55]	135.74 [33.94; 263.89]	0.294
	24 h after surgery	45, 3.25 [2.46; 4.81]	49, 3 [2.3; 3.91]	0.333
	% of change from baseline to 24 h	47, 79.31 [19.61; 182.45]	64.38 [18.28; 140.62]	0.636

Continuation of the Table 2.

Laboratory parameter		Values in groups		P^1
		IA, *N*=48	TIVA, *N*=50	
T lymphocytes (CD3+), %	Before surgery	47, 70.4 [64.9; 78.9]	70.75 [62.1; 78.1]	0.829
	1 hour after surgery	47, 67.5 [54.1; 74.9]	65.95 [54.7; 72.63]	0.549
	% change from baseline before 1 h	47, –8.17 [–18.65; 2.02]	–6.41 [–20.62; 1.2]	0.657
	24 h after surgery	47, 73.8 [65.5; 81.2]	71.9 [66.78; 77.58]	0.654
	% of change from baseline to 24 h	47, 1.62 [–4.19; 6.78]	2.39 [–5.57; 10.66]	0.945
T helpers (CD3+CD4+), %	Before surgery	47, 61.5 [53.9; 66.8]	59.75 [52.88; 71.13]	0.798
	1 hour after surgery	47, 61.1 [53.7; 68.2]	56.8 [43.58; 69.38]	0.292
	% change from baseline before 1 h	47, –1.16 [–6.47; 7.46]	–3.18 [–16; 3.78]	0.136
	24 h after surgery	47, 65.7 [56.7; 71.5]	63.4 [52.6; 72.23]	0.518
	% of change from baseline to 24 h	47, 4.64 [–0.83; 12.86]	3.88 [–3.77; 14.23]	0.675
Cytotoxic T lymphocytes (CD3+CD8+), %	Before surgery	47, 32 [25.3; 38.6]	32.65 [22.2; 39.68]	0.940
	1 hour after surgery	47, 31 [26; 37.6]	36 [22.8; 45.7]	0.392
	% change from baseline before 1 h	47, –2.54 [–10.58; 11.93]	4.39 [–6.4; 23.74]	0.075
	24 h after surgery	47, 27 [22.7; 36.2]	30.15 [21.63; 38.7]	0.433
	% of change from baseline to 24 h	47, –8.15 [–17.38; 0.3]	–5.86 [–14.87; 9.2]	0.375
Immuno-regulatory index (CD4+/CD8+ ratio)	Before surgery	46, 1.85 [1.49; 2.5]	1.66 [1.34; 3.22]	0.872
	1 hour after surgery	47, 1.95 [1.47; 2.52]	1.54 [0.97; 2.88]	0.203
	% change from baseline before 1 h	**46, 1.58 [–16; 18.4]**	**–8.15 [–35; 10.16]**	**0.033**
	24 h after surgery	45, 2.57 [1.61; 3.06]	49, 2.13 [1.36; 3.2]	0.394
	% of change from baseline to 24 h	45, 11.26 [–4.98; 36.06]	49, 15.87 [–9.2; 35.84]	0.907
B lymphocytes, %	Before surgery	47, 8.9 [7; 11.5]	9.65 [6.18; 13.18]	0.762
	1 hour after surgery	47, 10.7 [8.1; 14.3]	10.8 [7.68; 16.88]	0.991
	% change from baseline before 1 h	47, 25.41 [2.53; 44.83]	29.7 [–6.3; 44.39]	0.765
	24 h after surgery	47, 12.7 [8.5; 15.7]	49, 12.3 [9; 17.1]	0.484
	% of change from baseline to 24 h	47, 40.59 [9.01; 61.18]	49, 30.88 [9.31; 68.73]	0.498
NK cells, %	Before surgery	47, 16.2 [11.8; 23.4]	14.9 [8.63; 22.3]	0.191
	1 hour after surgery	47, 16 [10.3; 27.9]	17.5 [10.48; 26]	0.988
	% change from baseline before 1 h	47, 14.42 [–29.71; 65.87]	20.21 [–14.58; 83.06]	0.259
	24 h after surgery	47, 11.3 [8; 16.6]	48, 8.95 [6.43; 14.78]	0.189
	% of change from baseline to 24 h	47, –35.91 [–53.82; 0.78]	48, –35.93 [–52.71; 6.72]	0.615
T lymphocytes (CD3+), B lymphocytes (CD19+CD3), NK cells (CD3–CD16+), %	Before surgery	**46, 98.5 [96.53; 99.95]**	**97.2 [95.18; 98.6]**	**0.021**
	1 hour after surgery	47, 98.3 [95.5; 99.9]	97.25 [93.78; 99.23]	0.151
	% change from baseline before 1 h	46, –0.3 [–3.22; 2.56]	0.36 [–2.54; 2]	0.668
	24 h after surgery	45, 98.4 [96.6; 99.55]	48, 97.4 [94.3; 99.08]	0.105
	% of change from baseline to 24 h	44, –0.35 [–1.87; 1.89]	48, 0.07 [–2.31; 2.76]	0.487

Note. [1] — Mann–Whitney test; *Me* — median; *Q1* and *Q3* — first and third quartiles, respectively. Missing data are represented by the number of observations.

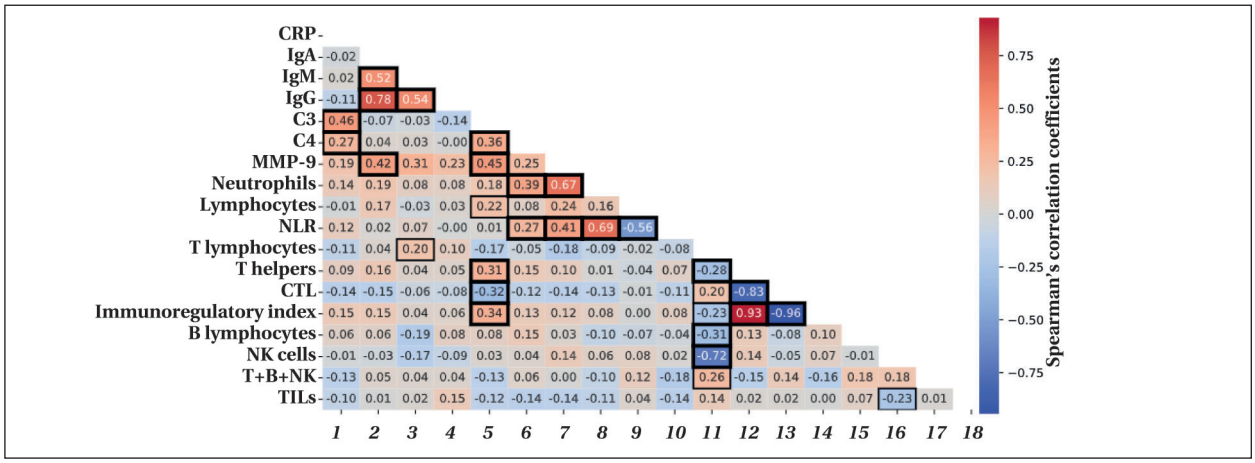

Fig. 2. Heat map of Spearman's correlation coefficients of preoperative (baseline) parameter values.
Note. *1* — CRP; *2* — IgA; *3* — IgM; *4* — IgG; *5* — C3; *6* — C4; *7* — MMP-9; *8* — Neutrophils; *9* — Lymphocytes; *10* — NLR; *11* — T lymphocytes; *12* — T helpers; *13* — CTL; *14* — Immunoregulatory index; *15* — B lymphocytes; *16* — NK cells; *17* — T+B+NK; *18* — TILS. Correlation is statistically significant for coefficients highlighted by a frame: thin frame at $P<0.05$, thick frame at $P<0.01$. CRP — C-reactive protein; MMP-9 — matrix metalloproteinase-9; NLR — neutrophil-lymphocyte ratio; CTL — cytotoxic lymphocytes; T+B+NK — total T, B lymphocytes and NK cells. TILs — tumor infiltrating lymphocytes.

system (C3, C4) ($P<0.001$; $R=0.36$). A strong positive significant correlation was found between MMP-9 and absolute neutrophil count ($P<0.001$; $R=0.673$), and a very strong negative correlation was found between the number of cytotoxic T cells and T helper cells ($P<0.001$; $R=–0.828$). NK cells showed a strong negative quantitative correlation with T cells ($P<0.001$; $R=–0.725$). No significant quantitative relationship was found between NK cells, T killers and neutrophils.

Detailed analysis results, including *P*-values and Spearman's correlation coefficients with their 95% confidence intervals, are available in the Supplementary Appendix at request from correspondence address.

Inhalation anesthesia

Changes at 1 hour. The results of the correlation analysis of the relative changes in the parameters at 1 hour after surgery in the IA group are presented as a heat map (Fig. 3, *a*).

All immunoglobulins showed a very strong positive correlation ($P<0.001$):

- IgA–IgG: $R=0.928$
- IgA–IgM: $R=0.837$
- IgG–IgM: $R=0.815$

This was accompanied by a concurrent decrease in the levels of all immunoglobulins.

The levels of complement system components (C3 and C4) also decreased. A strong positive correlation was observed between these components ($P<0.001$; $R=0.782$).

There was a weak positive correlation between the levels of complement system components and immunoglobulins, with statistically significant associations characterized by *P* values ranging from 0.011 to 0.023 and *R* values ranging from 0.313 to 0.363.

Thus, all components of the humoral immune system showed consistent changes (suppression) with varying degrees of correlation strength.

A significant positive moderate correlation was observed between T killers, NK cells and neutrophils, with R values ranging from 0.447 to 0.503 ($P\leqslant0.002$), as well as a moderate negative correlation between T helpers and NK cells ($P<0.001$; $R=–0.578$).

All significant correlations between components of the humoral immune system (IgA, IgM, IgG, C3, C4) and cells involved in «foreign body clearance» (T killers, NK cells, neutrophils) were positive, with strengths ranging from weak to moderate (*P* values between 0.003 and 0.049; *R* values between 0.291 and 0.427). A concomitant change in humoral immunity and the number of cells in the «foreign body clearance» system was observed.

The level of C-reactive protein (CRP) correlated positively with components of humoral immunity (IgA, IgM, IgG, C3, C4), as well as with the number of T killers, NK cells and neutrophils, with correlations ranging from weak to moderate ($P<0.001$ to 0.031; *R* values from 0.248 to 0.566).

Changes at 24 hours. The results of the correlation analysis of the relative changes in parameters 24 hours after surgery in the IA group are presented as a heat map (Fig 3, *b*).

The pattern and direction of changes in immunoglobulins and components of the complement system at 24 hours remained consistent with those observed at the previous measurement. The levels of all components of the humoral immune system continued to decrease, with varying degrees of correlation.

A strong positive correlation was observed between the number of T killers and NK cells ($P<0.001$; $R=0.743$), accompanied by a shift of the values to the lower range. Neutrophils no longer correlated quantitatively with T killers. A strong negative correlation remained between T helpers and T killers.

The relationship between humoral and cellular immunity disappeared. CRP level was no longer associated with other parameters.

Total intravenous anesthesia

Changes at 1 hour. The results of the correlation analysis of the relative changes in parameters at 1 hour after surgery in the TIVA group are presented as a heat map (Fig. 3, *c*).

All immunoglobulins showed strong or very strong positive correlations ($P<0.001$; *R* ranged from 0.802 to 0.907). This was accompanied by a concomitant increase in the levels of all immunoglobulins.

A moderate positive correlation was observed between the levels of complement components C3 and C4 ($P<0.001$; $R=0.596$). Plasma concentrations of these proteins 1 hour after surgery were lower than their baseline levels.

No significant correlations were found in five of six pairwise comparisons between complement components (C3, C4) and immunoglobulins (IgA, IgM, IgG). For one pair (IgA and C3), a weak positive correlation was observed ($P=0.039$; $R=0.293$).

Overall, different components of the humoral immune system responded in divergent directions: immunoglobulin concentrations increased simultaneously, whereas components of the complement system decreased in a coordinated manner. However, no significant correlations were observed between the parameters of these systems.

A strong positive correlation was observed between the number of T killers and NK cells ($P<0.001$; $R=0.722$), with a corresponding increase in the number of T killers in patient plasma. Neutrophils did not show a significant quantitative relationship with NK cells or T killers. However, a strong negative correlation was observed between NK cells and T helpers ($P<0.001$; $R=–0.759$).

For most pairwise comparisons between humoral immunity components (IgA, IgM, IgG, C3, C4) and cells involved in «foreign body clearance»

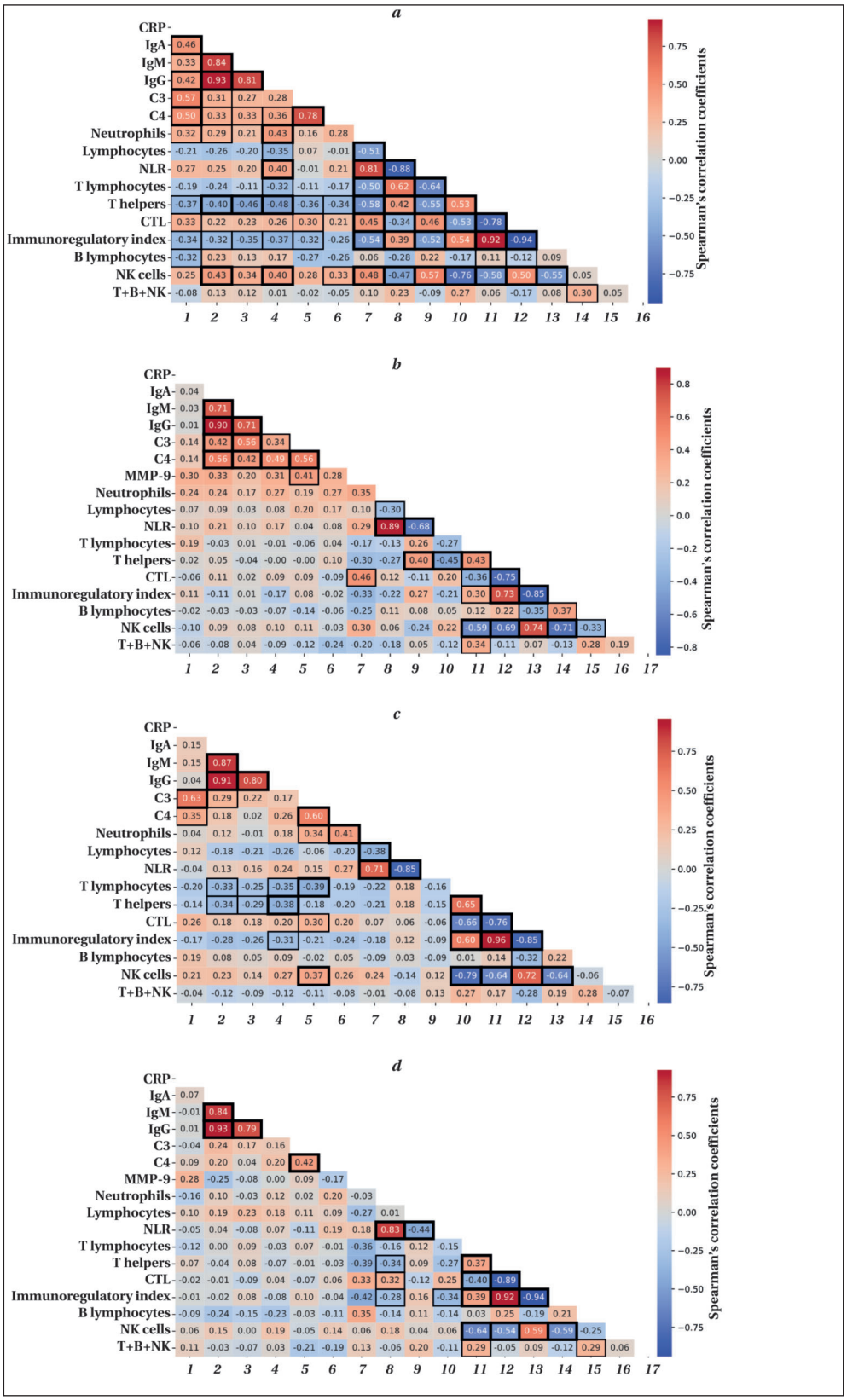

Fig. 3. Heatmaps of Spearman's correlation coefficients for relative changes in parameters at 1 hour (*a, c*) and 24 hours (*b, d*) in the inhalation anesthesia (IA; *a, b*) and total intravenous anesthesia (TIVA; *c, d*) groups.

Note. *1* — CRP; *2* — IgA; *3* — IgM; *4* — IgG; *5* — C3; *6* — C4; *7* — MMP-9; *8* — Neutrophils; *9* — Lymphocytes; *10* — NLR; *11* — T lymphocytes; *12* — T helpers; *13* — CTL; *14* — Immunoregulatory index; *15* — B lymphocytes; *16* — NK cells; *17* — T+B+NK. Correlation is statistically significant for coefficients highlighted by a frame: thin at $P<0.05$, thick at $P<0.01$. CRP — C-reactive protein; MMP-9 — matrix metalloproteinase-9; NLR — neutrophil-lymphocyte ratio; CTL — cytotoxic lymphocytes; T+B+NK — total T, B lymphocytes and NK cells. TILs — tumor infiltrating lymphocytes.

(T killers, NK cells, neutrophils), no significant correlations were identified. For a small subset of parameters (3 out of 15 pairs), weak positive correlations were found (P values ranging from 0.007 to 0.034; R values ranging from 0.301 to 0.374). In addition, neutrophils demonstrated a moderate correlation with C4 (P=0.003; R=0.415).

Overall, no concurrent changes between humoral and cellular immunity were observed in this group.

CRP levels did not correlate with markers of cellular immunity or immunoglobulin levels. However, a moderate positive association was observed with components of the complement system (with C3: $P<0.001$; R=0.63; with C4: P=0.012; R=0.354).

Changes at 24 hours. The results of the correlation analysis of the relative parameter changes at 24 hours postoperatively in the TIVA group are presented as a heat map (Fig. 3, *d*).

Immunoglobulin levels showed strong to very strong positive correlations with each other ($P<0.001$; R ranged from 0.786 to 0.926). Concurrently, their levels decreased.

Concentrations of complement system components (C3 and C4) increased and showed a moderate positive correlation (P=0.002; R=0.422).

No significant correlations were observed between complement proteins (C3, C4) and immunoglobulins (IgA, IgM, IgG). Thus, there was no systemic alignment in the changes of humoral immunity parameters.

A moderate positive correlation was observed between the number of T killers and NK cells ($P<0.001$; R=0.592), with an associated decrease in the number of T killers. Neutrophils showed a weak positive correlation with T killers (P=0.027; R=0.316), but not with NK cells. There was a moderate negative correlation between NK cells and T helpers.

No significant correlations were found between humoral and cellular markers of immunity.

CRP level was not associated with any immunologic parameter.

The correlations of the parameters in the IA and TIVA groups at 1 hour and 24 hours postoperatively are shown in Table 3.

Overall and recurrence-free survival. No recurrences or deaths were observed during the one-year follow-up period.

Discussion

In the IA group, strong correlations were observed between immunoglobulins (IgA, IgM, IgG) and complement system components (C3, C4), as well as their related dynamic changes.

Possible mechanisms underlying these effects include the direct influence of anesthetics on B lymphocytes, which are responsible for immunoglobulin production. Research suggests that anesthetics such as isoflurane and sevoflurane may suppress B lymphocyte function by modulating signaling pathways and inhibiting the transcription of genes essential for antibody synthesis [14]. In addition, elevated cortisol levels induced by anesthetics and surgical stress may affect the immune response by reducing antibody production through alterations in B lymphocyte activity [15]. The reduction in complement components (C3 and C4) may be due to their increased activation and subsequent depletion

Table 3. Comparison of correlations between parameters in the IA and TIVA groups at 1 hour and 24 hours postoperatively

Parameter	Values in groups							
	IA, *N*=48				TIVA, *N*=50			
	1 hour		24 hours		1 hour		24 hours	
	Correlation strength	Direction of change	Correlation strength	Direction of change	Correlation strength	Direction of change	Correlation strength	Direction of change
Immunoglobulins (IgA, IgM, IgG)	Very strong	↓↓	Strong/ very strong	↓↓	Strong/ very strong	↑↑	Strong/ very strong	↓↓
Complement systema (C3, C4)	Strong	↓↓	Moderate	↓↓	Moderate	↓↓	Moderate	↑↑
Humoral immunity	Weak	↓↓	Weak/ moderate	↓↓	5/6 no, 1 — weak (IgA and C3)	↑↑ (C3↓)	No	
T killers, NK cells, neutrophils	Moderate	↑↑ (T killers↓)	Strong (T killers and NK cells)	↓↓ (Neutrophils↑)	Strong (T killers and NK cells)	↑↑	Weak/ moderate	↓↓ (Neutrophils↑)
Humoral and cellular immunity	Weak/ moderate	↓↓(except NK cells and neutrophils)	No		No or Weak		No	
CRP with humoral and cellular immunity	Weak/ moderate	↓↓	No		Moderate with the complement system components	↓↓	No	

Note. Unidirectional arrows indicate positive correlation, bidirectional arrows indicate negative correlation.

in response to systemic inflammation associated with inhalation anesthetics. Alternatively, it may reflect the potential suppressive effects of anesthetics on liver function, which plays a critical role in protein synthesis [16]. The observed correlations between complement components and immunoglobulins highlight their joint contribution to humoral immunity and underscore the importance of a comprehensive investigation of the mechanisms underlying their interactions.

These findings differ from those of A. L. Kvarnström et al. who studied patients undergoing colorectal surgery and reported significant increases in C3a and SC5b-9 levels both intraoperatively and postoperatively in both the IA and TIVA groups. Such discrepancies underscore the significant role of surgical stress in complement activation. In their study, peak C3a levels occurred during surgery and remained elevated 24 hours postoperatively, suggesting a prolonged effect of surgical stress on the complement system [17].

Given the current lack of clinical studies demonstrating humoral immune suppression caused by reduced immunoglobulin levels under the influence of inhalational anesthetics, the findings presented here require further validation.

The strong negative correlation between NK cells and T-helper cells may reflect a form of «competition» between the innate and adaptive immune systems. NK cells, central players in innate immunity, have the ability to eliminate tumor cells without prior activation, although their functionality and efficiency can be significantly enhanced by specific cytokines. In contrast, T cells, essential components of adaptive immunity, require full activation to perform their functions. This divergent behavior may be explained by competition for limited resources, such as cytokines and growth factors, which could lead to suppression of one cell population while enhancing the activity of another [18, 19].

Literature suggests that both NK cells and T helper cells are dependent on IL-2 and IL-15 for activation. Imbalances in these cytokines could lead to preferential activation of one cell type at the expense of the other [20]. In addition, exposure to anesthetics can disrupt the production of IL-2 and other cytokines, potentially altering immune dynamics to favor NK cell activity over T cell responses — or vice versa — depending on the prevailing cytokine milieu [21].

Under normal conditions, T helper cells play a crucial role in the activation and support of cytotoxic T cells. By promoting the proliferation and differentiation of cytotoxic T cells, they ensure an effective immune response against tumor cells. However, exposure to inhalation anesthetics may suppress T helper cell function, thereby reducing the cytotoxic activity of CD8+ cells. This imbalance in the immune response could account for diminished antitumor activity and an increased risk of disease recurrence [22].

While the precise immune system targets of inhalation anesthetics remain undefined, several molecular and cellular mechanisms underlying their immunomodulatory effects have been identified. These mechanisms include the reduction of immune cell numbers through apoptosis and the suppression of cellular immune functions [16]. Given the complexity and variability of immune responses, the immunosuppressive effects of anesthetics are likely more intricate than previously recognized. Further clinical research, incorporating detailed analyses of blood immune parameters, is essential to clarify the mechanisms by which inhalation anesthetics modulate the immune system and their interrelated effects.

The weak correlations observed between humoral immune components (IgA, IgM, IgG, C3, C4) and cytotoxic immune cells (T killers, NK cells, neutrophils) suggest differing sensitivities of these immune subsystems to anesthetic exposure. This disparity may reflect distinct regulatory and activation mechanisms for humoral and cellular immunity. Moreover, individual variability in immune responses among patients likely contributes to the diverse reactions observed following anesthesia and surgical stress.

It seems that there was no single immunomodulatory factor in the TIVA group. At 1 hour postoperatively, some components of humoral immunity (immunoglobulins) increased in parallel, while other components (complement system) decreased. However, these changes were not related. At the same time, a subset of cellular immunity (T killers) was synchronously activated, resulting in an increased number of these cells 1 hour after surgery. By 24 hours postoperatively, components of humoral immunity (immunoglobulins and complement system) showed no correlation. The number of T killers decreased synchronously, but no associations were found between changes in cellular and humoral immunity.

Current literature suggests that propofol exerts less pronounced immunosuppressive effects compared to inhalation anesthetics. Specifically, propofol does not significantly suppress NK cell activity and has minimal effects on macrophage functions, including migration and polarization, thereby preserving immune function [23]. Despite the described immune changes, overall and recurrence-free survival at 1 year postoperatively was 100% in both the IA and TIVA groups.

The absence of mortality in this cohort is likely due to the low risk profile of patients with stage IA-IIA breast cancer (T1–2, N0, M0). However, the selection of this patient population inherently limits the external validity of the study and its applicability to long-term surgical outcomes.

The absence of adverse events may also be due to the limited sample size. Although post-hoc power calculations to assess overall and recurrence-free survival could not be performed due to the absence of recurrences and deaths, the low probability of these events in the study population combined with the small sample size highlights the limited power of the study to assess long-term outcomes, which is a major limitation.

Conclusion

Inhalation anesthesia was associated with synchronous changes in humoral immune components, whereas the total intravenous anesthesia resulted in divergent immune responses, suggesting potential differences in the effects of IA and TIVA on the immune system. However, no effect of anesthesia type on overall or recurrence-free survival was observed in patients with stage IA-IIA breast cancer during the one-year postoperative period. Further research is needed to elucidate the mechanisms by which different anesthetics influence immune status and to explore the relationship between anesthetic type and long-term oncologic outcomes.

References

1. *Hiller J. G., Perry N. J., Poulogiannis G., Riedel B., Sloan E. K.* Perioperative events influence cancer recurrence risk after surgery. *Nat Rev Clin Oncol*. 2018; 15 (4): 205–218. DOI: 10.1038/nrclinonc.2017.194. PMID: 29283170.
2. *Chang C.-Y., Wu M.-Y., Chien Y.-J., Su I.-M., Wang S.-C., Kao M.-C.* Anesthesia and long-term oncological outcomes: a systematic review and meta-analysis. *Anesth Analg*. 2021; 132 (3): 623–634. DOI: 10.1213/ANE.0000000000005237. PMID: 33105278.
3. *Степанов А. В., Шаповалов К. Г.* Мониторинг иммунной системы у пациентов в критическом состоянии (обзор). *Общая Реаниматология*. 2024; 20 (3): 42–52. *Stepanov A. V., Shapovalov K. G.* Monitoring the immune system in critically ill patients (review). *General Reanimatology = Obshchaya Reanimatologiya*. 2024; 20 (3): 42–52. (in Russ&Eng). DOI: 10.15360/1813-9779-2024-3-2388.
4. *Овечкин А. М., Политов М. Е., Сокологорский С. В., Евсюкова М. А.* Пропофол или ингаляционные анестетики: можно ли говорить о ренессансе тотальной внутривенной анестезии? *Анестезиология и Реаниматология*. 2021; (5): 71–79. *Ovechkin A. M., Politov M. E., Sokologorsky S. V., Evsyukova M. A.* Propofol vs inhalation anesthetics: can we talk about the renaissance of total intravenous anesthesia? *Russian Journal of Anaesthesiology and Reanimatology = Anesteziologiya i Reanimatologiya*. 2021; (5): 71–79. (in Russ.). DOI: 10.17116/ANAESTHESIOLOGY202105171.
5. *Sumi C., Matsuo Y., Kusunoki M., Shoji T., Uba T., Iwai T., Bono H., Hirota K.* Cancerous phenotypes associated with hypoxia-inducible factors are not influenced by the volatile anesthetic isoflurane in renal cell carcinoma. *PLoS One*. 2019; 14 (4): e0215072. DOI: 10.1371/journal.pone.0215072. PMID: 30986231.
6. *Efremov S. M., Kozireva V. S., Moroz G. B., Abubakirov M. N., Shkoda O. S., Shilova A. N., Yarmoshuk S. V., et al.* The immunosuppressive effects of volatile versus intravenous anesthesia combined with epidural analgesia on kidney cancer: a pilot randomized controlled trial. *Korean J Anesthesiol*. 2020; 73 (6): 525–533. DOI: 10.4097/kja.19461. PMID: 32098012.
7. *Cho J. S., Lee M.-H., Kim S. Il., Park S., Park H. S., Lee J. H., Koo B.-N.* The effects of perioperative anesthesia and analgesia on immune function in patients undergoing breast cancer resection: a prospective randomized study. *Int J Med Sci*. 2017; 14 (10): 970. PMID: 28924368.
8. *Buckley A., McQuaid S., Johnson P., Buggy D. J.* Effect of anaesthetic technique on the natural killer cell anti-tumour activity of serum from women undergoing breast cancer surgery: a pilot study. *Br J Anaesth*. 2014; 113 (suppl_1): i56–62. PMID: 25009196.
9. *Ситкин С. И.* Возможности ингаляционных анестетиков в блокировании чрезмерной воспалительной реакции: обзор литературы. *Вестник Интенсивной Терапии Имени А И Салтанова*. 2022; 2022 (3): 102–110. *Sitkin S. I.* Possibilities of inhalation anesthetics in blocking an excessive inflammatory response: a review. *Ann Crit Care = Vestnik Intensivnoy Terapii im AI Saltanova*. 2022; 2022 (3): 102–110. (in Russ). DOI: 10.21320/1818-474X-2022-3-102-110.
10. *Ackerman R. S., Luddy K. A., Icard B. E., Piñeiro Fernández J., Gatenby R. A., Muncey A. R.* The effects of anesthetics and perioperative medications on immune function: a narrative review. *Anesth Analg*. 2021; 133 (3): 676–689. DOI: 10.1213/ANE.0000000000005607. PMID: 34100781.
11. *Лихванцев В. В., Ландони Д., Субботин В. В., Каданцева К. К., Жукова Л. А., Ядгаров М. Я., Белетти А., с соавт.* Влияние выбора метода анестезии на иммунный ответ пациенток, перенесших радикальную операцию по поводу рака молочной железы (мета-анализ сравнительных клинических исследований). *Общая Реаниматология*. 2022; 18 (4): 20–28. *Likhvantsev V. V., Landoni D., Subbotin V. V., Kadantseva K. K., Zhukova L. A., Yadgarov M. Ya., Beletti A., et al.* Impact of anesthesia method on immune response in patients undergoing radical surgery for breast cancer (a meta-analysis of comparative clinical studies). *General Reanimatology = Obshchaya Reanimatologiya*. 2022; 18 (4): 20–28. (in Russ.&Eng.). DOI: 10.15360/1813-9779-2022-4-20-28.
12. *Kadantseva K., Subbotin V., Akchulpanov R., Berikashvili L., Yadgarov M., Zhukova L., Kvetenadze G., et al.* The impact of inhalation versus total intravenous anesthesia on the immune status in patients undergoing breast cancer surgery: a double-blind randomized clinical trial (TeMP). *Front Oncol*. 2024; 14. DOI: 10.3389/fonc.2024.1401910. PMID: 39132502.
13. Resources for Correlation > Spearmans Correlation Coefficient from statstutor n.d.: https://www.statstutor.ac.uk/topics/correlation/spearmans-correlation-coefficient/ (accessed July 29, 2024).
14. *Snyder G. L., Greenberg S.* Effect of anaesthetic technique and other perioperative factors on

cancer recurrence. *Br J Anaesth*. 2010; 105 (2): 106–115. PMID: 20627881.
15. *Rossaint J., Zarbock A.* Perioperative inflammation and its modulation by anesthetics. *Anesth Analg*. 2018; 126 (3): 1058–1067. DOI: 10.1213/ANE.0000000000002484. PMID: 28922235.
16. *Stollings L. M., Jia L.-J., Tang P., Dou H., Lu B., Xu Y.* Immune modulation by volatile nesthetics. *Anesthesiology*. 2016; 125 (2): 399–411. DOI: 10.1097/ALN.0000000000001195. PMID: 27286478.
17. *Kvarnström , Sarbinowski R. T., Bengtson J.-P., Jacobsson L. M., Bengtsson A. L.* Complement activation and interleukin response in major abdominal surgery. *Scand J Immunol*. 2012; 75 (5): 510–516. DOI: 10.1111/j.1365-3083.2012.02672.x. PMID: 22229650.
18. *Vivier E., Tomasello E., Baratin M., Walzer T., Ugolini S.* Functions of natural killer cells. *Nat Immunol*. 2008; 9 (5): 503–510. DOI: 10.1038/ni1582. PMID: 18425107.
19. *Griffith C. D., Kamath M. B.* Effect of halothane and nitrous oxide anaesthesia on natural killer lymphocytes from patients with benign and malignant breast disease. *Br J Anaesth*. 1986; 58 (5): 540–543. DOI: 10.1093/bja/58.5.540. PMID: 3964519.
20. *Ferlazzo G., Morandi B.* Cross-talks between natural killer cells and distinct subsets of dendritic cells. *Front Immunol*. 2014; 5: 159. DOI: 10.3389/fimmu.2014.00159. PMID: 24782864.
21. *Jafarzadeh A., Hadavi M., Hassanshahi G., Rezaeian M., Vazirinejad R.* General anesthetics on immune system cytokines: a narrative review article. *Anesthesiol Pain Med*. 2020; 10 (4): e103033. DOI: 10.5812/aapm.103033. PMID: 33134146.
22. *Chaudhry A., Rudra D., Treuting P., Samstein R. M., Liang Y., Kas A., Rudensky A. Y.* CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science*. 2009; 326 (5955): 986–991. DOI: 10.1126/science.1172702. PMID: 19797626.
23. *Perry N., Wigmore T.* Propofol (TIVA) versus volatile-based anesthetics: is there any oncological benefit? *Curr Anesthesiol Rep*. 2018; 8. DOI: 10.1007/s40140-018-0296-z.

Received 19.09.2024
Accepted 27.11.2024

https://doi.org/10.15360/1813-9779-2025-1-28-37

Perioperative Neuroprotection with Systemic Hypothermia During Carotid Endarterectomy

Alexey A. Syrovatsky[1]*, Ionas S. Simutis[1,2], Alexey V. Svetlikov[1,3], Konstantin M. Lebedinsky[2,4], Alexey N. Shcheglov[5], Vyacheslav A. Ratnikov[1], Daria E. Reznichek[2], Evgenia V. Khaldina[2]

[1] Sokolov Northwestern District Research and Clinical Center, Federal Medico-Biological Agency of Russia, 4 Ave. Culture, 194291 Saint Petersburg, Russia
[2] I. I. Mechnikov North-Western State Medical University, Ministry of Health of Russia, 47 Piskarevskii prospect, 195067 St. Petersburg, Russia
[3] St. Petersburg State University, 7–9 Universitetskaya nab., 199034 St. Petersburg, Russia
[4] Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[5] Central Clinical Hospital with Outpatient, Russian Presidential Administration Department, 15 Marshala Timoshenko Str., 121359 Moscow, Russia

For citation: *Alexey A. Syrovatsky, Ionas S. Simutis, Alexey V. Svetlikov, Konstantin M. Lebedinsky, Alexey N. Shcheglov, Vyacheslav A. Ratnikov, Daria E. Reznichek, Evgenia V. Khaldina.* Perioperative Neuroprotection with Systemic Hypothermia During Carotid Endarterectomy. *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 28–37. https://doi.org/10.15360/1813-9779-2025-1-28-37 [In Russ. and Engl.]

***Correspondence to:** Alexey A. Syrovatsky, drsyrovatskiy@yandex.ru

Summary

Prevention of brain injury during carotid endarterectomy (CEA) remains a significant challenge. Moderate controlled systemic hypothermia may serve as a potential neuroprotective measure during these procedures.

Aim of the study. To investigate the neuroprotective effects of moderate systemic hypothermia during CEA.

Materials and methods. Fifty-nine patients undergoing CEA under combined anesthesia were included. Patients were divided into two groups: the hypothermia group (N=33) and the normothermia control group (N=26). Both groups received standard measures to prevent cerebral ischemia. The hypothermia group received additional moderate systemic hypothermia aimed at a temperature range of 34–35°C. Cognitive function was assessed preoperatively and at 2, 5, and 30 days postoperatively using neurocognitive tests. Statistical analysis was performed with IBM SPSS Statistics.

Results. The incidence of cognitive impairment was 21.1% in the hypothermia group and 26.9% in the normothermia group. Postoperative cognitive impairment was more common in the normothermia group: 15.38% on day 5 and 11.5% on day 30 postoperatively compared to 12.1% and 6.1% in the hypothermia group (P<0.05).

Conclusion. This study demonstrated the neuroprotective effects of hypothermia, manifested by a reduced severity of cognitive impairment in the hypothermia group. Further research is needed to identify high-risk patients who would benefit most from this neuroprotective strategy and to optimize hypothermia protocols.

Keywords: neuroprotection by systemic hypothermia; therapeutic hypothermia; moderate hypothermia; carotid endarterectomy

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

Information about the authors:
Alexey A. Syrovatsky: ORCID 0000-0002-4768-8856
Ionas S. Simutis: ORCID 0000-0002-2537-0142
Alexey V. Svetlikov: ORCID 0000-0001-8652-8778
Konstantin M. Lebedinsky: ORCID 0000-0002-5752-4812
Alexey N. Shcheglov: ORCID 0000-0002-3783-7918
Vyacheslav A. Ratnikov: ORCID 0000-0002-9645-8408
Daria E. Reznichek: ORCID 0009-0001-6040-5144
Evgenia V. Khaldina: ORCID 0009-0007-2815-0616

Introduction

Carotid artery stenosis accounts for 20–40% of all strokes [1–3]. Carotid endarterectomy (CEA) remains the primary surgical intervention for secondary stroke prevention [4, 5]. However, this procedure is not without risk and may independently contribute to cerebrovascular complications, including transient or permanent neurological deficits. This necessitates efforts to minimize surgical risks and improve procedural safety [4, 5].

Beyond acute cerebrovascular events, there are less severe but equally concerning postoperative outcomes, such as cognitive dysfunction, that significantly impact patients' quality of life. Current data suggest that up to 25% of patients, and possibly more in some reports, experience postoperative

cognitive dysfunction (POCD) following CEA [6]. The etiology of these disorders is multifactorial, often involving intraoperative factors. These may include intraoperative ischemia, microembolism, transient hypoperfusion, or reperfusion injury after restoration of blood flow to the internal carotid artery [6]. A combination of these factors is often observed.

A large meta-analysis of cognitive dysfunction after CEA, which included 60 studies and 4.823 cases, showed an association between the development of cognitive impairment and evidence of hypo- and hyperperfusion during the procedure. Furthermore, cognitive dysfunction was more common in patients with prolonged internal carotid artery clamping, highlighting the role of surgical factors in its development [7].

Preventing and treating cognitive dysfunction during the perioperative period, regardless of its etiology, remains a critical responsibility of anesthesiologists [8, 9].

Extensive experience has been gained in clinical practice worldwide in the use of various methods to protect the brain from ischemia and hypoperfusion. These include maintaining elevated arterial blood pressure during internal carotid artery (ICA) clamping, ensuring a high fraction of oxygen in the ventilated gas mixture (especially during general anesthesia with mechanical ventilation), placement of temporary intraluminal shunts, and the use of metabolic neuroprotective agents, among others [10]. However, these methods do not guarantee protection against brain injury and in some cases may even increase its likelihood [11].

A potential additional measure in the neuroprotective armamentarium is controlled moderate hypothermia. The therapeutic effects of hypothermia have been well documented in areas such as neonatal hypoxic-ischemic encephalopathy, post-cardiac arrest syndrome, and as part of comprehensive ischemic stroke therapy using local hypothermia [12, 13]. Several mechanisms underlying the neuroprotective effects of cooling in such contexts have been discussed [14]. Reports have highlighted the successful application of local cerebral hypothermia in reducing brain injury volume in ischemic stroke [13]. In addition, this method of neuroprotection has been studied in carotid endarterectomy, where it has been shown to affect brain metabolism during unilateral ICA occlusion. However, the use of cerebral hypothermia devices during surgery can be challenging due to procedural technical limitations [15].

To address intraoperative neuroprotection in carotid surgery, the use of controlled systemic hypothermia within a temperature range of 34.0°C to 35.0°C appears promising [16]. However, data on its protective properties remain inconsistent [17, 18]. Furthermore, neither the optimal hypothermia protocol nor its duration to achieve maximal neuroprotective effects has been definitively established. It is possible that the beneficial neuroprotective effects of hypothermia in previous studies were offset by confounding factors related to the depth of hypothermia, its complexity and/or invasiveness, and other methodological nuances. These considerations highlight the need for further research.

Materials and Methods

The effectiveness of systemic hypothermia was evaluated at the L. G. Sokolov North-Western District Scientific and Clinical Center (Sokolov NWDSCC, St. Petersburg) from September 2022 to November 2023. A pilot, single-center, randomized trial was conducted and approved by the local ethics committee of the Sokolov NWDSCC (LEC protocol No. 6 dated August 22, 2022).

The inclusion, exclusion, and post-randomization withdrawal criteria are shown in Table 1.

Based on the above criteria, 59 patients who underwent surgery for atherosclerosis of the brachiocephalic vessels were included in the study. The preliminary sample size calculation was performed using the following formula:

$$n = \frac{z^2 p(1-p)}{E^2}$$

where:

— n is the calculated sample size;

— z is the confidence coefficient;

— p is the expected proportion of patients with postoperative cognitive impairment;

— E is the margin of error.

For the calculation, the z coefficient for standard conditions (with a 95% confidence level) was set at 1.96. The expected proportion of patients with postoperative cognitive impairment was 15% (the expected rate based on the available literature at the time of the final screening). The margin of error used was 5%.

The initial result was then adjusted for the population of up to 100 patients expected to be screened during the study period using the following formula:

$$n = \frac{n_0}{1 + (n_0 - 1)/N}$$

where:

— n_0 is the sample size calculated using the previous formula;

— N is the population size;

— n is the adjusted sample size for the population.

The population was limited to the estimated number of patients eligible for screening within the study period.

As a result of the calculation, the sample size was determined to be 67 patients.

The primary endpoint of the study was the incidence of cognitive impairment in the groups. Secondary endpoints included the development of stroke, length of hospital stay, and mortality. None of these outcomes were observed in the enrolled patients. The endpoint used for calculation was the incidence of cognitive impairment in the groups.

All patients underwent carotid endarterectomy. Eight patients were excluded: three patients withdrew from the study, four refused cognitive testing, and one patient was discharged on the day of surgery due to the development of an acute respiratory illness. Patients were randomized into two groups using an envelope method: the main group, which underwent moderate systemic hypothermia (*N*=33), and the control group (*N*=26), in which normothermia was maintained without any temperature-maintaining measures. Opaque envelopes and double-blinding were used for randomization. Block randomization was not used. The enrollment process is shown in Fig. 1.

Group characteristics are shown in Table 2.

Anesthesia and pharmacologic agents used during anesthesia may contribute to cognitive decline [19, 20]. Therefore, all patients underwent carotid endarterectomy under combined anesthesia: cervical plexus block with 0.5% ropivacaine under ultrasound guidance combined with general anesthesia. Induction of general anesthesia was performed routinely in both groups with fentanyl (1.5–2.0 μg/kg), propofol (1.5–2.0 mg/kg), and muscle relaxation with rocuronium (0.6–0.8 mg/kg). Maintenance anesthesia was achieved with sevoflurane (0.8–1.0 MAC) or propofol at 3–4 mg/kg/h. The choice of maintenance anesthetic was left to the anesthesiologist. The study did not use double randomization.

Because hypothermia can affect the pharmacokinetics of propofol and muscle relaxants, their doses were adjusted in the main group based on data from the depth of anesthesia monitor and neuromuscular transmission monitoring.

During the intraoperative period, in addition to the Harvard standard for monitoring, the following methods were used: invasive arterial blood pressure measurement, depth of sedation monitoring (GE Healthcare M-Entropy), neuromuscular transmission monitoring (NMT GE Healthcare), registration of cerebral oximetry data using near-infrared spectroscopy (Somanetics Invos), and assessment of acid-base balance and blood gas parameters at all stages of hypothermia (before initiation, at target level, and before rewarming). Coagulation status (activated coagulation time, ACT) and mechanical ventilation parameters were also monitored. For thermometry, temperature sensors were placed in the retropericardial segment of the esophagus and in the axillary region.

Patient awakening and recovery from anesthesia were assessed using the Aldrete scale. The main characteristics of surgery and anesthesia in both groups are shown in Table 3.

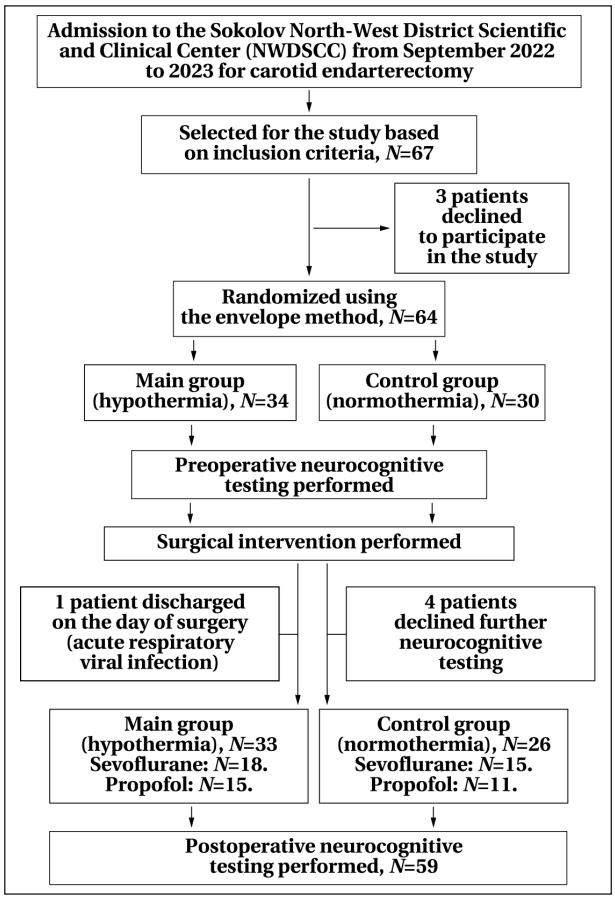

Fig. 1. Flowchart of Patient Selection in the Study.

Table 1. Criteria for Patient Selection in the Study.

Criteria of		
inclusion	**exclusion**	**post-randomization exclusion**
1. Patients with atherosclerosis of the brachiocephalic arteries (BCA) indicated for carotid endarterectomy. 2. Men and women over 18 years of age. 3. Patient consent to participate in the study.	1. Individuals unable to understand the goals and objectives of the study. 2. Patients with severe neurological deficits precluding neurocognitive testing. 3. Patients with terminal stages of chronic diseases. 4. Patients with critical ischemia of the lower extremities. 5. Patients with BMI greater than 30. 6. Patients participating in other studies. 7. Use of pharmacological neuroprotectors.	1. Withdrawal of informed consent by the patient. 2. Refusal to participate in further neurocognitive testing stages.

Table 2. Characteristics of patient groupsв, *Me (Q25–Q75)* or *N* (%).

Parameter	Values in the groups					*P*-value
	Hypothermia, *N*=33		Normothermia, *N*=26		Z-score*, *N*=30	
	Propofol	Sevoflurane	Propofol	Sevoflurane		
Age, years	69.5 (64.75–75.5)	68 (65.5–75)	71 (69–75.5)	68.5 (64–73)	69 (61–71.5)	0.659
Sex						
male	10 (66.67)	13 (72.22)	7 (63.64)	10 (66.67)	17 (56.67)	0.860
female	5 (33.33)	5 (27.78)	4 (36.36)	5 (33.33)	13 (43.33)	
Smoker	6 (40)	10 (55.56)	5 (45.45)	9 (60)	18 (60)	0.708
Stroke/TIA	5 (33.33)	6 (33.33)	4 (36.36)	5 (33.33)	7 (23.33)	0.898
Diabetes mellitus	3 (20)	3 (16.67)	2 (18.18)	4 (26.67)	9 (30)	0.825
Hypertension	13 (86.67)	17 (94.44)	9 (81.82)	12 (80)	25 (83.33)	0.782
Hyperlipidemia	4 (26.67)	6 (33.33)	5 (45.45)	9 (60)	13 (43.33)	0.402
CHD	10 (66.67)	9 (50)	6 (54.54)	6 (40)	11 (36.67)	0.383
Arrhythmias	2 (13.33)	1 (5.56)	0	3 (20)	4 (13.33)	0.510
Symptomatic stenosis	4 (26.67)	6 (33.33)	5 (45.45)	4 (26.67)	1 (3.33)	0.026
Neurological deficit	2 (13.33)	3 (16.67)	1 (9.09)	3 (20)	0	0.203
Overweight	2 (13.33)	1 (5.56)	1 (9.09)	2 (13.33)	10 (33.33)	0.106

Note. * — patients not undergoing surgery or anesthesia, matched for age and testing interval to patients in the normothermia and hypothermia groups.

Description of the hypothermia technique. In the main group, after induction of anesthesia and placement of temperature sensors in the retropericardial segment of the esophagus and the axillary region, controlled hypothermia was initiated using the Hypoterm device (Medmos, Russia) with a target core temperature of 34.0–35.0°C. Cooling was performed with a heat-exchange mattress and blanket. The hypothermia device was set to the coolant and target cooling temperatures, and the cooling process was stopped when the target temperature was reached. The temperature was then maintained within the target range. In most cases, the target cooling temperature was reached by the time the carotid artery was clamped. Hypothermia was maintained until the internal carotid clamp was removed. Monitoring parameters during cooling (core and peripheral body temperatures, coolant temperature, adverse events) were recorded in the protocol every 10 minutes. The hypothermia technique was based on established national and international practices [12, 21].

The rewarming process was then initiated with a target temperature of 36.0°C. Due to the inertia of the cooling process, rewarming did not begin immediately, and the rate of rewarming was limited to no more than 0.5°C per hour. After surgery, rewarming was continued in the intensive care unit using convective warming devices.

In both groups, cerebral protection against hypoperfusion was performed using routine methods described in the literature: maintaining systemic arterial pressure at 20–25% above baseline, increasing the oxygen concentration in the gas mixture to FiO_2 0.9, and placing a temporary intraluminal shunt when cerebral oxygenation fell below 15% of baseline. In equivocal cases, additional monitoring of retrograde pressure in the internal carotid artery stump was performed [11, 10]. Adverse events related to hypothermia were recorded intraoperatively and postoperatively (e. g., coagulation disorders, electrolyte imbalances, arrhythmias, postoperative shivering, perioperative infectious complications) according to the ESAIC perioperative complications list [22].

Cognitive function was assessed using standard scales: MMSE, MOCA, and TMT (Trail Making Test) at baseline and on postoperative days 2, 5, and 30. The assessments were performed by an vascular

Table 3. Surgery and anesthesia characteristics, *Me (Q25–Q75)* or *N* (%).

Parameter	Values in groups						*P**
	Hypothermia, *N*=33			Normothermia, *N*=26			
	Total	Sevoflurane	Propofol	Total	Sevoflurane	Propofol	
Duration of surgery, min	130 (107.5–145)	135 (115–159)	125 (110–132)	120 (111.3–135)	120 (105–145)	120 (115–140)	0.738
Duration of anesthesia, min	220 (145–236.3)	225 (175–245)	205 (170–212.5)	200 (177.5–210)	195 (145–227.5)	192.5 (176.25–207.5)	0.289
Duration of internal carotid artery clamping, min	53 (38.8–61.3)	60 (45–72.5)	40 (40–50)	40 (40–55)	50 (35–57.5)	45 (40-55)	0.257
The use of temporary intraluminal shunt	3 (9.09)	2 (13.33)	1 (9.09)	3 (11.54)	1 (5.56)	2 (13.33)	0.655
Classical carotid endarterectomy technique	3 (9.09)	1 (6.67)	2 (18.18)	3 (11.54)	2 (11.11)	1 (6.67)	0.705
Eversion carotid endarterectomy technique	30 (90.91)	17 (94.44)	13 (86.67)	23 (88.46)	13 (86.67)	10 (90.91)	0.299
Blood loss, mL	40 (30–50)	50 (35–55)	50 (35–55)	40 (30–50)	40 (27.5–50)	40 (30–50)	0.523
Aldrete score	9 (8–9.3)	9 (8.5–9.5)	9 (8.5–10)	9 (8–10)	9 (8–9.5)	9 (8–10)	0.894

Note. * — Intergroup comparison based on the total values in the «Total» column.

neurologist who was unaware of the patient's group assignment.

Because cognitive assessment tests have different dimensions, Z-scores were used to compare test results [23]. A third group (Z-score, *N*=30) of patients in the same age range who had not undergone surgery or anesthesia was tested at the same intervals as the normothermia and hypothermia groups.

The standardized Z-score for each patient was calculated from the raw test result using the formula:

$$Z = \frac{x - X}{SD}$$

where:

x is the raw test result for a given patient,

X is the mean,

SD is the standard deviation for the particular test.

Postoperative cognitive impairment (POCI) was defined as Z-scores that differed from baseline by –1.96 SD or more on at least two tests. If these changes persisted for more than 30 days, the patient was considered to have postoperative cognitive dysfunction (POCD). If detected earlier, it was classified as delayed neurocognitive recovery [24].

Statistical Analysis. Data were analyzed using IBM SPSS 26 with nonparametric statistical methods. Results were presented as medians (Me) and interquartile ranges *(Q25–Q75)*. The Kruskal–Wallis test was used to compare quantitative variables between groups, and the Mann–Whitney *U* test was used for pairwise comparisons of two independent groups. Pearson's χ^2 test was used for categorical variables. A $P<0.05$ was considered statistically significant. A two-tailed *P*-value was used.

Results and Discussion

The groups were homogeneous with respect to baseline characteristics. Among the patients studied, 24 (41%) had right carotid stenosis and 35 (59%) had left carotid stenosis.

The most common comorbidities in this cohort were arterial hypertension (86.4%), coronary artery disease (57.6%), and diabetes mellitus (20.3%). In the main and control groups, 10 and 9 patients, respectively, had a history of transient ischemic attack or stroke in the operated artery territory.

The study results demonstrated that systemic cooling to a target temperature of 34.0–35.0°C, initiated prior to carotid clamping and maintained during clamping, is technically feasible and safe.

There was no statistically significant difference in the duration of surgery and carotid clamping time between the groups: 120 (111.3–135) minutes in the control group and 130 (107.5–145) minutes in the main group. Carotid artery occlusion time was 53 (38.8–61.3) minutes in the main group and 40 (40–55) minutes in the control group (*P*=0.78). There was no significant difference in the dosage and duration of sympathomimetic support between the groups. Mean arterial pressure during carotid occlusion was maintained above baseline in both groups, taking into account cerebral oximetry data. None of the patients experienced infectious complications while receiving the standard regimen of antibiotic prophylaxis.

One patient in the main group and one in the control group experienced paroxysmal atrial fibrillation (AF) in the early post-operative period, both had a history of paroxysmal AF. One patient in the control group required prolonged norepinephrine administration due to persistent hypotension, but the need for vasopressors was resolved within the first few hours. Five patients in the overall cohort required narcotic analgesia during the first postoperative day. The rest of the patients received analgesia with nonsteroidal anti-inflammatory drugs.

Overall, no significant differences were found between the groups based on standard criteria for perioperative complications. However, specific characteristics related to hypothermia were noted.

The incidence of postoperative cognitive impairment (POCI) is shown in Fig. 2. These impairments mainly manifested as delayed neurocognitive recovery. On postoperative day 2, the incidence of POCI was 21.2% in the hypothermia group and 26.9% in the normothermia group. POCI were observed more frequently in the normothermia group, occurring in 15.4% of patients on day 5 and 11.5% on day 30, compared to 12.1% and 6.1%, respectively, in the hypothermia group ($P<0.05$).

One month after surgery, improvement in cognitive function was observed in 4 patients (12.1%) in the hypothermia group, while only 1 patient (3.8%) in the normothermia group showed improvement.

Within each group, the effects of anesthetic maintenance agents (sevoflurane or propofol) were

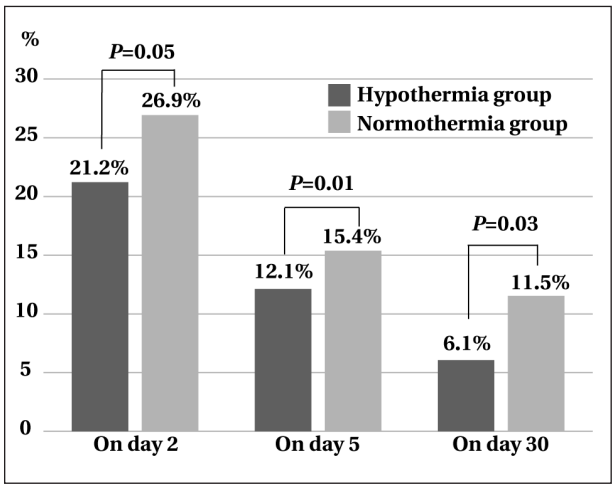

Fig. 2. Frequency of postoperative cognitive impairment.

also analyzed. A trend toward a higher incidence of cognitive impairment was observed in patients receiving sevoflurane. In the hypothermia group, POCI developed in 2 patients receiving sevoflurane, whereas no cognitive impairment was observed in patients receiving propofol. A similar trend was observed in the normothermia group: POCI was identified in 2 of 15 patients receiving sevoflurane compared to 1 of 11 patients receiving propofol.

The results obtained regarding the safety of moderate hypothermia during carotid endarterectomy are consistent with the existing literature. For example, S. Candela et al. [15] cooled patients to 34.5–35.0°C using an invasive thermoregulation device. The authors concluded that hypothermia within this temperature range does not cause clinically significant adverse effects and that shivering can be effectively managed with medication. Similar conclusions were reached by the authors of studies on the use of «mild» hypothermia in neurosurgery [25].

However, in the study by M. Todd et al., which focused on the use of therapeutic hypothermia during surgery for clipping cerebral aneurysms, a higher incidence of bacteremia was reported in the hypothermia group, along with a more frequent need for prolonged mechanical ventilation due to insufficient rewarming after the hypothermic phase. However, it should be noted that their cooling protocol reached 33.5°C, which is below the target range in our study.

Controlled hypothermia is a relatively simple method of neuroprotection during carotid endarterectomy. The duration of the procedure eliminates the risk of an excessively long hypothermic period, thereby minimizing potential adverse effects.

Overall, the incidence of POCI observed in this study is comparable to data reported by other investigators. According to a meta-analysis by P. Aceto et al. on cognitive impairment after CEA, the overall incidence of POCI was 14.1%, with delayed cognitive recovery occurring in 20.5% of cases. However, in individual studies included in the analysis, this rate was higher, reaching up to 45% [26].

It is noteworthy that the frequency of POCI one month after CEA reported in this study is lower than in some other studies. For example, in K. Relander's study, cognitive deficits persisted in 44% of patients three months after surgery [26]. This high detection rate of cognitive dysfunction may be due to the use of a more extensive battery of neuropsychological tests. Similarly, in a study by T. Klypa on POCI in cardiac surgery patients, varying degrees of cognitive dysfunction were observed in 30–70% of patients in the early postoperative period using a battery of four tests [27].

Undoubtedly, cognitive impairment in this patient population is not only influenced by intraoperative risk factors. Additional contributing factors may include suboptimal pharmacological treatment of atherosclerosis and comorbidities, progression of other serious systemic diseases, preexisting central nervous system disease, and others [26]. However, based on the results of this study, it can be assumed that intraoperative risks and their potential modification contribute significantly to treatment outcomes.

The use of hypothermic neuroprotection during surgery, in combination with other conventional methods of protecting the brain from injury, was found to reduce the severity of cognitive impairment compared with the normothermia group. In the normothermia group, delayed neurocognitive recovery was more common in asymptomatic patients than in symptomatic patients. In the hypothermia group, however, no such differences were observed between symptomatic and asymptomatic patients.

In a study by Trae R. Robison et al., the authors found no significant differences in the incidence of cognitive impairment between symptomatic and asymptomatic patients. However, they highlighted that the beneficial effects of statins and aspirin on the development of POCI were particularly pronounced in asymptomatic patients. This suggests that asymptomatic patients may be particularly vulnerable and, as such, may require enhanced neuroprotection. This is particularly important considering that surgical treatment of asymptomatic patients tends to yield better results in preventing ischemic events compared to symptomatic patients [28, 29].

A trend toward increased incidence of POCI was observed with the use of sevoflurane in both groups. Although our data did not reach statistical significance due to the small sample size, they are consistent with the findings of other investigators. For example, a meta-analysis of 34 studies involving 4.314 elderly patients reported a postoperative neurocognitive disorder rate of 16.8% in the propofol group and 24.0% in the sevoflurane group [31].

According to the literature, shivering is the most common adverse effect observed during controlled hypothermia [30], and this was clinically confirmed in our study. During the early postoperative period, shivering was observed in 4 patients (12%) immediately after awakening. Of these 4 patients, 2 received excessive cooling (i. e., beyond the target temperature range) during surgery (down to 33.5°C), which prolonged the rewarming process. The excessive cooling was attributed to the inherent inertia of the cooling method used. No other serious adverse effects related to hypothermia were observed.

The literature describes the use of meperidine, magnesium sulfate, and tramadol to lower the shivering threshold [30]. As shivering can have potentially adverse effects on the patient, it was promptly

treated by slow intravenous administration of a 25% magnesium sulfate solution.

The issue of rewarming patients after hypothermia deserves special attention. Some investigators in their studies did not awaken patients and extubate the trachea until normothermia was achieved. In particular, M. Todd et al. awakened patients in the ICU several hours later [25]. This approach may have contributed to a higher incidence of infectious complications in their observations. However, other authors report that mild hypothermia within the specified temperature range is well tolerated by patients without anesthesia: discomfort caused by this regimen can be alleviated with moderate sedation and anti-shivering medications [30].

In a review by S. Inoue on the use of therapeutic hypothermia during the intraoperative period, the author concludes that extubation can be performed in the operating room if there are no other contraindications. If necessary, sedation should be continued until the patient is fully rewarmed [21]. Similarly, we proceeded with awakening and tracheal extubation while gradually rewarming the patient, without waiting for normothermia to be fully restored. The residual sedation remaining in patients after anesthesia helped to mitigate any potential subjective discomfort caused by hypothermia. In addition, a key consideration in rewarming is to prevent the development of hyperthermia in the postoperative period. Even mild hyperthermia significantly exacerbates ischemic brain injury and can potentially worsen neurocognitive test scores [13].

The low incidence of adverse events was likely related to both the hypothermic protocol and the short duration of its use. In addition, the small surgical site and lack of significant blood loss during the procedure made the hypothermic process well controlled, avoiding excessive cooling or prolonged rewarming in most cases.

The lack of a registered protocol in a clinical registry is a limitation of the study. Nevertheless, to minimize the risk of subjective modifications and interpretations, the protocol approved prior to the clinical trial was strictly followed. Out of 64 randomized patients, neurocognitive testing was not performed in 5 cases, which could have influenced the assessment of the severity of POCI.

Conclusion

Controlled hypothermia is a safe and easily reproducible neuroprotective method in carotid endarterectomy with a minimal number of adverse effects.

References

1. *Верещагин Н. В., Пирадов М. А., Суслина З. А.* Инсульт. Принципы диагностики, лечения и профилактики. Краткое руководство для врачей. Москва: Интермедика; 2002: 208. *Vereshchagin N. V., Piradov M. A., Suslina Z. A.* Stroke. Principles of diagnosis, treatment and prevention. A short guide for physicians. Moscow: Intermedica, 2002: 208. (in Russ.).
2. *Grotta J. C.* Clinical practice. Carotid stenosis. *N Engl J Med*. 2013; 369 (12): 1143–1150. DOI: 10.1056/NEJMcp1214999. PMID: 24047063.
3. *Rerkasem A., Orrapin S., Howard D. P., Rerkasem K.* Carotid endarterectomy for symptomatic carotid stenosis. *Cochrane Database Syst Rev*. 2020; 9 (9): CD001081. DOI: 10.1002/14651858.CD001081.pub4. PMID: 32918282.
4. North American Symptomatic Carotid Endarterectomy Trial Collaborators; *Barnett H. J. M., Taylor D. W., Haynes R. B., Sackett D. L., Peerless S. J., Ferguson G. G., Fox A. J., et al.* Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med*. 1991; 325 (7): 445–453. DOI: 10.1056/NEJM199108153250701. PMID: 1852179.
5. Endarterectomy for asymptomatic carotid artery stenosis. Executive committee for the asymptomatic carotid atherosclerosis study. JAMA. 1995; 273 (18): 1421–1428. PMID: 7723155.
6. *Heyer E. J., Gold M. I., Kirby E. W., Zurica J., Mitchell E., Halazun H. J., Teverbaugh L., et al.* A study of cognitive dysfunction in patients having carotid endarterectomy performed with regional anesthesia. *Anesth Analg*. 2008; 107 (2): 636–642. DOI: 10.1213/ane.0b013e3181770d84. PMID: 18633045.
7. *Aceto P., Lai C., De Crescenzo F., Crea M. A., Di Franco V., Pellicano G. R., Perilli V., et al.* Cognitive decline after carotid endarterectomy: Systematic review and meta-analysis. *Eur J Anaesthesiol*. 2020; 37 (11): 1066–1074. DOI: 10.1097/EJA.0000000000001130. PMID: 31860600.
8. *Александрович Ю. С., Акименко Т. И., Пшениснов К. В.* Послеоперационная когнитивная дисфункция — является ли она проблемой для анестезиолога-реаниматолога? *Вестник анестезиологии и реаниматологии*. 2019; 16 (4): 5–11. *Alexandrovich Y. S., Akimenko T. I., Pshenisnov K. V.* Postoperative cognitive dysfunction — is it a problem for the anesthesiologist-resuscitator? *Messenger of Anesthesiology and Resuscitation = Vestnik Anesthesiologii i Reanimatologii*. 2019; 16 (4): 5–11. (in Russ.). DOI: 10.21292/2078-5658-2019-16-4-5-11.
9. *Щеголев А. В., Лахин Р. Е., Кучина С. Н., Спивак Д. Л., Шаповалов И. С., Спивак И. М.* Влияние метода анестезии на послеоперационные когнитивные нарушения при многократных оперативных вмешательствах у пациентов с тяжелой сочетанной травмой: пилотное исследование. *Анестезиология и реаниматология*. 2023; 6: 29–35. *Shchegolev A. V., Lakhin R. E., Kuchina S. N., Spivak D. L., Shapovalov I. S., Spivak I. M.* Influence of anesthetic technique on postoperative cognitive impairment following multiple surgical interventions in patients with severe concomitant injury: a pilot study. *Russian Journal of Anaesthesiology and Reanimatology = Anesteziologiya i Reanimatologiya*. 2023; 6: 29–35. (in Russ.). DOI: 10.17116/anaesthesiology202306129.
10. *Howell S. J.* Carotid endarterectomy. *Br J Anaesth*. 2007; 99 (1): 119–131. DOI: 10.1093/bja/aem137. PMID: 17556351.
11. *Simon M. V., Malcharek M., Ulkatan S.* Monitoring in carotid endarterectomy. *Handb Clin Neurol*. 2022; 186: 355–374. DOI: 10.1016/B978-0-12-819826-1.00015-6. PMID: 35772895.
12. *Шевелев О. А., Петрова М. В., Саидов Ш. Х., Ходорович Н. А., Прадхан П.* Механизмы нейропротекции при церебральной гипотермии (обзор). *Общая реаниматология*. 2019; 15 (6): 94–114. *Shevelev O. A., Petrova M. V., Saidov Sh. Kh., Khodorovich N. A., Pradkhan P.* Neuroprotection mechanisms in cerebral hypothermia (review). *General Reanimatology = Obshchaya Reanimatologiya*. 2019; 15 (6): 94–114. (in Russ.&Eng.). DOI: 10.15360/1813-9779-2019-6-94-114.
13. *Задворнов А. А., Григорьев Е. В.* Целевое управление температурой у новорожденных при проведении общей терапевтической гипотермии. *Анестезиология и реаниматология*. 2022; (3): 5557. *Zadvornov A. A., Grigoriev E. V.* Targeted temperature management in neonates during general therapeutic hypothermia. *Russian Journal of Anaesthesiology and Reanimatology = Anesteziologiya i Reanimatologiya*. 2022; (3): 5557. (in Russ.). DOI: 10.17116/anaesthesiology202203155.
14. *Ивкин А. А., Балахнин Д. Г., Борисенко Д. В., Григорьев Е. В.* Возможности церебропротекции у детей в кардиохирургии (обзор литературы). *Вестник анестезиологии и реаниматологии*. 2023; 20 (1): 89–96. *Ivkin A. A., Balakhnin D. G., Borisenko D. V., Grigoriev E. V.* Opportunities of cerebroprotection in children in cardiac surgery (review of literature). *Messenger of Anesthesiology and Re-*

suscitation/Vestnik Anesthesiologii i Reanimatologii. 2023; 20 (1): 89–96. (in Russ.).
DOI: 10.24884/2078-5658-2023-20-1-89-96.

15. *Чернявский А. М., Ломиворотов В. Н., Дерягин М. Н., Струнин О. В., Столяров М. С.* Кислородное обеспечение головного мозга в условиях краниоцеребрального охлаждения на этапах операции каротидной эндартерэктомии. *Патология кровообращения и кардиохирургия*. 2003; 2: 38–41. *Chernyavsky A. M., Lomivorotov V. N., Deryagin M. N., Strunin O. V., Stolyarov M. S.* Cerebral oxygen supply in craniocerebral cooling at stages of carotid endarterectomy surgery. *Pathology of Blood Circulation and Cardiac Surgery = Patologiya Krovoobrashcheniya i Kardiokhirurgiya*. 2003; 2: 38–41. (in Russ.).
16. *Candela S., Dito R., Casolla B., Silvestri E., Sette G., Filippi F, Taurino M., et al.* Hypothermia during carotid endarterectomy: a safety study. *PLoS One*. 2016; 11 (4): e0152658.
DOI: 10.1371/journal.pone.0152658.
PMID: 27058874.
17. *Garrido C. C., Gallego B. R. García J. C.S., Martín J. C., Troya M. M., Blanque R. R.* The effect of therapeutic hypothermia after cardiac arrest on the neurological outcome and survival-a systematic review of RCTs published between 2016 and 2020. *Int J Environ Res Public Health*. 2021; 18 (22): 11817.
DOI: 10.3390/ijerph182211817. PMID: 34831572.
18. *Habibi V., Habibi M. R., Habibi A., Zeydi A. E.* The protective effect of hypothermia on postoperative cognitive deficit may be attenuated by prolonged coronary artery bypass time: meta-analysis and meta-regression. *Adv Clin Exp Med*. 2020; 29 (10): 1211–1219.
DOI: 10.17219/acem/121920. PMID: 33064380.
19. *Луговой А. В., Пантелеева М. В., Надькина Е. Д., Овезов А. М.* Интраоперационная профилактика когнитивных нарушений при тотальной внутривенной анестезии у детей школьного возраста: рандомизированное клиническое исследование. *Вестник интенсивной терапии имени А.И. Салтанова*. 2018; 4: 57–64. *Lugovoy A. V., Panteleeva M. V., Nadkina E. D., Ovezov A. M.* Intraoperative prevention of cognitive impairment in total intravenous anesthesia in school-age children: randomized clinical trial. *Ann Crit Care = Vestnik Intensivnoy Terapii im AI Saltanova* 2018; 4: 57–64.
20. *Сыроватский А. А., Сайганов С. А., Лебединский К. М.* Мотивы выбора метода анестезии при выполнении каротидной эндартерэктомии. *Анестезиология и реаниматология*. 2022; 2: 7379. *Syrovatskiy A. A., Saiganov S. A., Lebedinsky K. M.* Anesthesia technique choice motives for carotid endarterectomy. *Russian Journal of Anaesthesiology and Reanimatology = Anesteziologiya i Reanimatologiya*. 2022; 2: 7379. (in Russ.).
DOI: 10.17116/anaesthesiology202202173.
21. *Inoue S.* Temperature management for deliberate mild hypothermia during neurosurgical procedures. *Fukushima J Med Sci*. 2022; 68 (3): 143–151.
DOI: 10.5387/fms.2022-28. PMID: 36517037.
22. *Jammer I., Wickboldt N., Sander M., Smith A., Schultz M. J., Pelosi P., Leva B., et al.* Standards for definitions and use of outcome measures for clinical effectiveness research in perioperative medicine: European Perioperative Clinical Outcome (EPCO) definitions: a statement from the ESA-ESICM joint taskforce on perioperative outcome measures. *Eur J Anaesthesiol*. 2015; 32 (2): 88–105.
DOI: 10.1097/EJA.0000000000000118.
PMID: 25058504.
23. *Субботин В. В., Душин И. Н., Камнев С. А., Антипов А. Ю.* Некоторые аспекты формирования Z-счета для оценки когнитивных расстройств. *Вестник анестезиологии и реаниматологии*. 2020; 17 (5): 25–30. *Subbotin V. V., Dushin I. N., Kamnev S. A., Antipov A. Yu.* Certain aspects of using Z-score to assess cognitive disorders. *Messenger of Anesthesiology and Resuscitation/Vestnik Anesthesiologii i Reanimatologii* 2020; 17 (5): 25–30. (in Russ.).
DOI: 10.21292/2078-5658-2020-17-5-25-30.
24. *Evered L., Silbert B., Knopman D. S., Scott D. A., DeKosky S. T., Rasmussen L. S., Oh E S., et al; Nomenclature Consensus Working Group.* Recommendations for the nomenclature of cognitive change associated with anaesthesia and surgery-2018. *Br J Anaesth*. 2018; 121 (5): 1005–1012.
DOI: 10.1016/j.bja.2017.11.087. PMID: 30336844.
25. *Todd M. M., Hindman B. J., Clarke W. R., Torner J., C.; Intraoperative Hypothermia for Aneurysm Surgery Trial (IHAST) Investigators.* Mild intraoperative hypothermia during surgery for intracranial aneurysm. *N Engl J Med*. 2005; 352 (2): 135–145. PMID: 15647576.
26. *Relander K., Hietanen M., Nuotio K., Ijäs P., Tikkala I., Saimanen E., Lindsberg P. J., et al.* Cognitive dysfunction and mortality after carotid endarterectomy. *Front Neurol*. 2021; 11: 593719.
DOI: 10.3389/fneur.2020.593719.
PMID: 33519678.
27. *Клыпа Т. В., Антонов И. О., Вавакин А. С.* Динамика когнитивного статуса кардиохирургических больных и предикторы его нарушения. *Анестезиология и реаниматология*. 2016; 61 (1): 18–23. *Klypa T. V., Antonov I. O., Vavakin A. S.* The cognitive status dynamics of cardiosurgical patients and the predictors of its disturbance. *Russian Journal of Anaesthesi-*

ology and Reanimatology = Anesteziologiya i Reanimatologiya. 2016; 61 (1): 18–23. (in Russ.). DOI 10.18821/0201-7563-2016-61-1-18-23.

28. *Прямиков А. Д., Миронков А. Б., Лолуев Р. Ю., Хрипун А. И.* Каротидная эндартерэктомия и стентирование внутренней сонной артерии у пациентов старших возрастных групп (обзор литературы). *Вопросы нейрохирургии имени Н. Н. Бурденко*. 2021; 85 (1): 113–117. *Pryamikov A. D., Mironkov A. B., Loluev R. Yu., Khripun A. I.* Carotid endarterectomy and carotid artery stenting in advanced age patients (literature review). *Burdenko's Journal of Neurosurgery = Voprosy Neirokhirurgii Imeni N. N. Burdenko*. 2021; 85 (1): 113–117. (in Russ.&Eng.). DOI: 10.17116/neiro202185011113.
29. *Oh E. C., Sridharan N. D., Avgerinos E. D.* Cognitive function after carotid endarterectomy in asymptomatic patients. *J Cardiovasc Surg (Torino)*. 2023; 64 (3): 317–321. DOI: 10.23736/S0021-9509.23.12632-2. PMID: 36897209.
30. *Jackson T. C., Kochanek P. M.* A new vision for therapeutic hypothermia in the era of targeted temperature management: a speculative synthesis. *Ther Hypothermia Temp Manag*. 2019; 9 (1): 13–47. DOI: 10.1089/ther.2019.0001. PMID: 30802174.
31. *Zeng K., Long J., Li Y., Hu J.* Preventing postoperative cognitive dysfunction using anesthetic drugs in elderly patients undergoing noncardiac surgery: a systematic review and meta-analysis. 2023; 109 (1): 21–31. DOI: 10.1097/JS9.0000000000000001. PMID: 36799783.

Received 09.08.2024
Accepted 28.12.2024

https://doi.org/10.15360/1813-9779-2025-1-38-48

Efficacy of Scoring Systems for Routing and Predicting Length of ICU Stay in Severe Community-Acquired Pneumonia

Irina A. Ruslyakova*, Elvina Z. Shamsutdinova, Galina A. Mityuchenko, Alexandra O. Orlova, Elena B. Avalueva

I. I. Mechnikov North-Western State Medical University, Ministry of Health of Russia, 47 Piskarevskii prospect, 195067 St. Petersburg, Russia

For citation: *Irina A. Ruslyakova, Elvina Z. Shamsutdinova, Galina A. Mityuchenko, Alexandra O. Orlova, Elena B. Avalueva.* Efficacy of Scoring Systems for Routing and Predicting Length of ICU Stay in Severe Community-Acquired Pneumonia. *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 38–48. https://doi.org/10.15360/1813-9779-2025-1-38-48 [In Russ. and Engl.]

***Correspondence to:** Irina A. Ruslyakova, ruslyakova777dok@gmail.com

Summary

Scoring systems based on assessment of disease severity and patient condition are widely used for routing and predicting length of stay in the ICU. However, their effectiveness varies in patients with sepsis.

The aim of the study. To evaluate the effectiveness of scoring systems in routing and predicting ICU length of stay in patients with severe community-acquired pneumonia (CAP).

Materials and methods. Medical records of 664 patients from the Intensive Care for Severe CAP database of I. I. Mechnikov Northwestern State Medical University (2013–2023) were analyzed using the following scoring scales: CURB-65, PSI/PORT, SMART-COP, SCAP, REA-ICU, NEWS2, IDSA/ATS criteria, APACHE IV, CFS, and CCI. Statistical analysis was performed using Statistica 10.0, SPSS, and Stat Research (Center for Statistical Research) software.

Results. Among the study cohort, 96 patients (15%) had bacterial severe CAP (bCAP) and 568 patients (85%) had viral severe CAP (vCAP), all of whom were admitted to the ICU. A NEWS2 score ⩾2 was observed in 74 (77.1%) bCAP patients and all vCAP patients ($P<0.001$). In contrast, 437 (76.9%) vCAP patients and 74 (77.1%) bCAP patients were classified as high risk according to SMART-COP ($P=0.966$). Delayed ICU admission (>7 days) was observed in older patients with severe bCAP, but did not significantly affect ICU length of stay or outcomes. A strong correlation was found between adverse outcome and predicted mortality using APACHE IV ($\eta=0.966$ for vCAP and $\eta=0.807$ for bCAP). However, no correlation was observed between predicted and actual ICU length of stay for both vCAP and bCAP patients ($R^2=0.0257$, Kendall's $W=0.018$ for vCAP; $R^2=0.0294$, Kendall's $W=0.050$ for bCAP). The predictive model accuracy for ICU stay >1 day or >14 days was not satisfactory. Model with vCAP patients adjusted for age (⩾60 years) and APACHE IV exhibited moderate predictive accuracy for prolonged ICU stay (AUROC 0.610).

Conclusion. Differences were found in the applicability of the NEWS2, REA-ICU, and IDSA/ATS major criteria scoring systems for ICU routing of bCAP and vCAP patients. APACHE IV showed a strong correlation between predicted and actual mortality, but no correlation between predicted and actual ICU length of stay in severe CAP patients was found.

Keywords: community-acquired pneumonia; ICU routing; ICU length of stay; severity scoring systems

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

Information about the authors:
Irina A. Ruslyakova: ORCID 0000-0003-1507-833X
Elvina Z. Shamsutdinova: ORCID 0009-0003-5800-8697
Galina A. Mityuchenko: ORCID 0009-0000-2047-9563
Alexandra O. Orlova: ORCID 0009-0000-1428-2617
Elena B. Avalueva: ORCID 0000-0001-6011-0998

Introduction

Community-acquired pneumonia (CAP) is the second most common cause of hospitalization and the leading infectious cause of death. Advanced age is a well-established risk factor for adverse outcomes in CAP [1]. Severe community-acquired pneumonia (sCAP) is a distinct form of the disease characterized by severe acute respiratory failure (ARF), typically accompanied by signs of sepsis and organ dysfunction [2]. A pragmatic definition of sCAP refers to CAP in patients admitted to the intensive care unit (ICU). The bias inherent in this definition stems from the significant variability in ICU resources across regions and healthcare institutions. In addition, the comorbidity profile of patients [1] and the need for specialized care in «frail» patients [3] may influence the assessment of severity and increase the need for ICU admission in CAP cases.

The use of severity scales and the selection of appropriate care settings are critical to ensure the safety of patients with CAP and the appropriate allocation of hospital resources (Appendix, Table). In clinical practice, the use of the IDSA/ATS minor criteria (3 out of 9 criteria) or major criteria (shock or need for mechanical ventilation) helps to stratify patients with CAP [4]. A meta-analysis by Marti et

al. showed that the minor criteria of IDSA/ATS, SCAP, and SMART-COP have superior discriminatory performance compared to the PSI/PORT and CURB-65 scoring systems in predicting the need for ICU admission in patients with sCAP [5]. Similar findings were reported in the study by Fukuyama et al, where the IDSA/ATS criteria and the SMART-COP scale showed good predictive value for ICU routing in patients with sCAP [6].

The proportion of patients with severe community-acquired pneumonia (sCAP) requiring resuscitation is 22.7% [7], and a proportion of these patients needs a prolonged stay in the intensive care unit (ICU) [8–9], which significantly increases the financial cost to hospitals [10]. Prolonged ICU stays are not only associated with high costs due to intensive therapy, but also with resource utilization, leading to disruptions in the throughput capacity of units and hospitals.

To predict ICU length of stay, the predictive ability of the APACHE III [11], APACHE IV [12], MPM III [13], and SAPS II [14] scales was evaluated. The APACHE IV scale was developed in 2006 using data from 69.652 patients admitted to 104 ICUs in the United States and later validated using data from 46.517 patients to predict ICU length of stay and hospital mortality [12]. The accuracy of predicting ICU length of stay using the APACHE IV scale in patients with sepsis shows conflicting results [15].

Building a predictive model by incorporating additional variables based on the APACHE II, APACHE III, and SAPS II scales shows higher effectiveness in predicting the risk of prolonged ICU stay (AUC 0.827–0.839) [16]. To improve the predictive accuracy in patients with sCAP, it is suggested to use the Clinical Frailty Scale (CFS) and the Charlson Comorbidity Index (CCI). According to several researchers, the CFS provides valuable clinical information for health care managers regarding the organization and duration of intensive therapy [3], and outcome prediction based on age and comorbidities using the CCI outperforms the CURB-65 and PSI/PORT scales in terms of accuracy in patients with sCAP [17].

Improving the accuracy of predicting ICU length of stay for patients with sCAP will facilitate planning and improve resource management in hospitals.

The aim of the study — to evaluate the effectiveness of using scales for patient routing and prediction of ICU length of stay in patients with severe community-acquired pneumonia.

Materials and Methods

Data from the medical records of 853 patients with lower respiratory tract infections were collected from the «Intensive care of patients with severe community-acquired pneumonia» database at the I. I. Mechnikov Northwestern State Medical University (NSMU) from February 2013 to February 2023 (government registration certificate for the database No. 2024624611). The diagnosis of severe community-acquired pneumonia (sCAP) was made according to clinical guidelines [2]. The center received approval from the Local Ethics Committee (LEC) of NSMU (LEC Protocol No. 2, dated February 12, 2020).

The table in the appendix shows the scales used prospectively to assess the severity of patients with sCAP and to determine the need for ICU admission. A retrospective assessment of 40 patients with moderate community-acquired pneumonia (mCAP) from 2013 to 2020 was performed using the NEWS2 scale. The duration of ICU stay for patients with sCAP was also evaluated. During ICU admission, variables necessary to predict mortality and ICU length of stay were collected.

Statistical analysis was performed using the software packages Statistica 10.0, SPSS, and Stat Research (Center for Statistical Research). Patient characteristics were compared between groups according to the distribution of quantitative variables. The Shapiro–Wilk test was used to assess normality. Quantitative data were described as median *(Me)* and interquartile range *(Q1; Q3)* or mean *(M)* ± standard deviation *(SD)*.

Independent groups were compared using the Mann–Whitney and Kruskal–Wallis tests, while paired samples were analyzed using the Wilcoxon test. The Bonferroni correction was used for multiple comparisons. The structure of categorical variables was presented as frequency distributions, and Pearson's χ^2 test was used for comparative analysis of categorical data. Statistical significance was set at a two-tailed $P<0.05$.

The association between quantitative variables was assessed using Spearman's rank correlation coefficient, concordance was assessed using Kendall's *W* coefficient, and the association between binary and continuous variables was measured using the eta (η) coefficient.

ROC analysis was used to assess the discriminative power of the scales. The optimal threshold was selected based on a balance between sensitivity and specificity. The results were reported as threshold, sensitivity, specificity, and area under the ROC curve (AUC). Model quality was graded as follows:

— 0.9–1.0 — excellent
— 0.8–0.9 — very good
— 0.7–0.8 — good
— 0.6–0.7 — moderate
— 0.5–0.6 — poor

A higher AUC indicates a greater prognostic (diagnostic) value of the scale.

Results and Discussion

The study flowchart is shown in Fig. 1.

The study included medical record data from 664 patients, of whom 96 (15%) had bacterial severe community-acquired pneumonia (bsCAP) and 568 (85%) had viral severe community-acquired pneumonia (vsCAP). The patient groups were comparable in age and gender, with older and geriatric patients predominant in both cohorts.

Patients with bsCAP had a higher Charlson Comorbidity Index (CCI) score, indicating a greater comorbidity burden, and their Clinical Frailty Scale (CFS) scores suggested a need for personalized care due to significant physical and cognitive impairment. Scores on the NEWS2, SMART-COP, REA-ICU scales and IDSA/ATS criteria were elevated in both groups, but showed differences between vsCAP and bsCAP patients.

A total of 567 (99.8%) vsCAP patients and 80 (83.3%) bsCAP patients required respiratory support of various modalities ($P<0.001$). However, the bsCAP group had a lower PaO_2/FiO_2 ratio. Among vsCAP patients, 203 (35.7%) had a bacterial coinfection on admission to the ICU.

The need for vasopressor support and the doses administered were higher in the bsCAP group. The groups were comparable in the use of corticosteroids but differed in the frequency of parenteral nutrition and administration of «last-resort antibiotics». Parenteral nutrition was used more frequently in vsCAP patients, while «last-resort antibiotics» were prescribed more frequently in bsCAP patients.

Patients with bsCAP required a longer ICU stay, while vsCAP patients had a longer overall hospital stay. A comparison of demographic, clinical, laboratory, and hospital-related parameters between the groups is shown in Table 1.

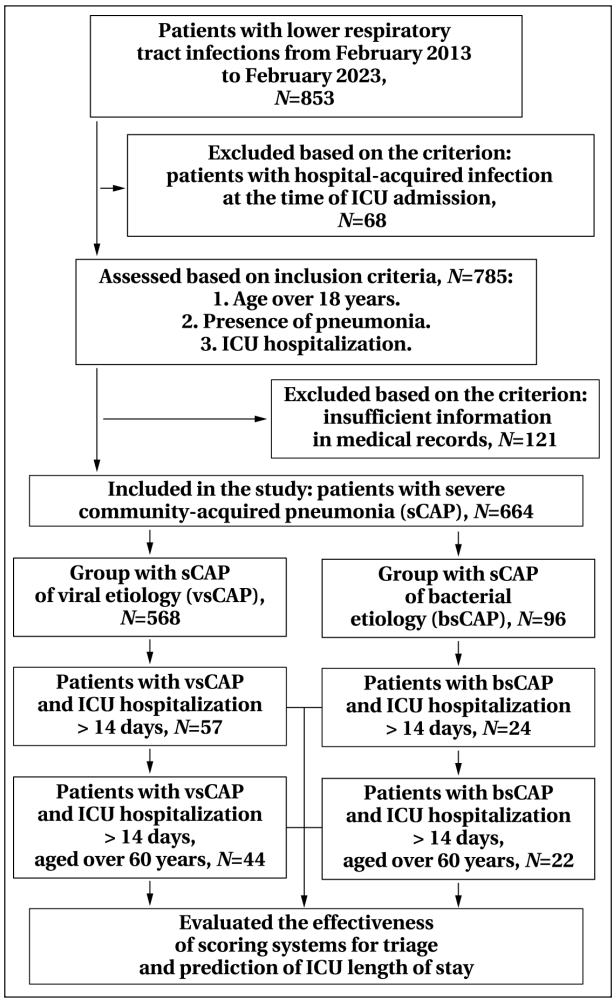

Fig. 1. Study flowchart.
Note. AUROC — area under the ROC curve; ROC — receiver operating characteristic.

In the study by Covino et al, the NEWS2 score showed a high predictive accuracy (AUROC 0.901) for ICU admission and/or mortality within 24 hours with a score ⩾ 2 [29], which is consistent with our findings, where 568 (100%) of patients with vsCAP and 74 (77.1%) with bsCAP were at high risk for ICU admission. In the study by Lazar Neto et al. of patients with community-acquired pneumonia hospitalized with COVID-19, a SMART-COP score ⩾ 3 was observed in 437 (76.9%) patients [30], which is consistent with our results [6, 18].

The SCAP scale, with a threshold > 10.0 points, also showed a high efficacy in predicting ICU admission in patients with vsCAP and bsCAP. A comparative analysis by Marti et al. showed that the IDSA/ATS minor criteria, SCAP score, and SMART-COP score had better discriminatory properties than PSI/PORT and CURB-65 for predicting ICU admission [5], which is consistent with our results. A PSI/PORT score > 130 (class V) was observed in 365 (64.2%) patients with vsCAP versus 53 (55.2%) with bsCAP, higher than in the similar study by Charles et al. However, the number of patients with a SMART-COP score ⩾ 3 was lower than in the study by P. G. Charles et al. [18].

The SCAP and REA-ICU scales showed differences between vsCAP and bsCAP patients requiring intensive care, as did the IDSA/ATS major criteria. Liapikou et al. showed that the predictive value of the IDSA/ATS criteria for ICU admission was 71% [31]. In the study by Renaud et al, the risk of ICU admission increased significantly from risk class I (⩽ 3 points) to risk class IV (⩾ 9 points) on the REA-ICU scale [20], similar to the data of the current study.

The threshold values of the scales for ICU admission and the number of patients in the severe CAP groups reaching the corresponding scores at ICU admission are shown in Table 2.

Seventy-three (76%) patients with bsCAP were admitted to the ICU within 48 hours vs. 266 (46.8%) patients with typical vsCAP. Delayed ICU admission was associated with increased hospital mortality and unplanned readmissions in both groups, although these differences between groups were not

Table 1. Demographic, clinical, laboratory, and hospitalization-related parameters in the study groups.

Parameter	Values in groups		*P*
	vsCAP	bsCAP	
Total, *N* (%)	568 (85.5)	96 (14.5)	
Demographic parameters			
Age, years	67.14±14.02 68 (58.5–78)	70.07±13.96 70 (61.5–82)	0.053
Elderly, *N* (%)	232 (40.85)	39 (40.62)	0.297
Senile, *N* (%)	173 (30.46)	37 (38.54)	
Middle-aged, *N* (%)	110 (19.37)	12 (12.50)	
Young, *N* (%)	42 (7.39)	5 (5.21)	
Long-lived individuals, *N* (%)	11 (1.94)	3 (3.12)	
Women, *N* (%)	265 (46.6)	57 (59.4)	
BMI, kg/m^2, Me (IQR)	27.8 (8.16)	25.4 (8.56)	0.002
Scores and evaluation systems, *Me (Q1–Q3)*			
NEWS2, points	9 (8–10)	5 (2–7.5)	<0.001
CCI, points	3 (2–6)	7 (6–8)	<0.001
CFS, points	2 (0–5)	6 (0–7)	<0.001
SOFA	5 (4–7)	4 (3–6)	0.056
APACHE IV, points	69.00 (48.00–123.25)	99.00 (65.00–126.00)	<0.001
CURB-65, points	3 (2–3)	3 (2–3)	0.854
SMART-COP, points	4 (3–4)	5 (3–7)	<0.001
PSI/PORT, points	136.0 (120.5–150.5)	132.0 (114.25–156.0)	0.443
SCAP, points	11.0 (11.0–18.0)	12.0 (8.5–18.0)	0.137
IDSA/ATS: Minor criteria, points	2 (2–4)	2 (1–3)	<0.001
IDSA/ATS: Major criteria, points	0 (0–1)	1 (0–1)	0.013
REA-ICU, points	5 (2–12)	10 (7–12)	<0.001
Organ support			
Respiratory support, *N* (%)	567 (99.8)	80 (83.3)	<0.001
High-flow oxygen therapy, *N* (%)	409 (72.0)	14 (14.6)	<0.001
NILV, *N* (%)	427 (75. 2)	14 (14.6)	<0.001
MLV > 24 hours, *N* (%)	248 (43.7)	40 (41.7)	0.777
PaO_2/FiO_2, mmHg in patients on NILV/MLV, Me (IQR)	156 (35.0)	116 (62.5)	<0.001
Vasopressor support (norepinephrine at a dose of > 0.5 μg/kg/min), *N* (%)	129 (22.71)	55 (57.29)	<0.001
Vasopressor support >72 hours	85 (14.96)	55 (57.29)	<0.001
Renal replacement therapy, *N* (%)	58 (10.2)	36 (38.7)	<0.001
Intensive care			
«Last-resort antibiotics», *N* (%)	124 (21.8)	36 (37.5)	<0.001
Steroids, *N* (%)	220 (38.7)	39 (40.6)	0.006
Parenteral nutrition, *N*, (%)	201 (35.4)	30 (31.25)	<0.001
Length of hospitalization and outcomes			
ICU stay > 14 days, *N* (%)	57 (10.0)	24 (25.0)	<0.001
Days in ICU, Me (IQR)	5.0 (6.0)	7.0 (9.75)	0.001
Days from Hospital Admission to ICU, Me (IQR)	2.0 (5.0)	1.0 (1.0)	<0.001
Length of hospital stay, days, Me (IQR)	17.0 (14.0)	12.5 (13.75)	0.002
Fatal outcome, *N* (%)	236 (41.55)	58 (60.42)	0.001

Note. *Me* — median; *IQR* — interquartile range; BMI — body mass index; NILV — non-invasive lung ventilation; MLV — mechanical lung ventilation.

Table 2. Use of assessment systems to identify high-risk patients for ICU admission.

Assessment system, points	Frequency in groups, *N* (%)		*P*
	vsCAP, *N*=568	bsCAP, *N*=96	
1. CURB-65 ⩾3	291 (51.2)	50 (52.1)	0.877
2. PSI/PORT >130	365 (64.2)	53 (55.2)	0.092
3. SMART-COP ⩾3	437 (76.9)	74 (77.1)	0.966
4. IDSA/ ATS: Major criteria ⩾1	263 (46.3)	62 (64.6)	0.001
5. IDSA/ ATS: Minor criteria ⩾3	281 (49.5)	40 (41.7)	0.158
6. SCAP ⩾10	451 (79.4)	62 (64.6)	0.001
7. REA-ICU ⩾7	265 (46.7)	72 (75.0)	<0.001
8. NEWS2 ⩾2	568 (100.0)	74 (77.1)	<0.001

statistically significant. Similar findings were reported in a large British cohort study that showed worse outcomes with delayed ICU admission. Mortality was 46.3% for those admitted to the ICU within 2 days of hospital admission, rising to 50.4% for those admitted within 2–7 days and 57.6% for those admitted after 7 days [32]. When comparing patients based on ICU admission time, delayed ICU admission (> 7 days) was more common in older patients with vsCAP (P=0.026) and was associated with longer hospital stay (P<0.0001). However, it did not significantly affect ICU length of stay or outcome. Patients with bsCAP admitted to the ICU after a delay of > 7 days also had longer hospital stays (P=0.002), but no differences in ICU length of stay or outcome were observed between groups (Table 3).

Table 3. Study parameters at different time points prior to ICU admission.

Parameter	Values at different time points prior to ICU admission			*P*
	<48 hours	From 2 to 7 days	>7 days	
Patients with vsCAP, *N* (%)	266 (46.8)	191 (33.6)	111 (19.5)	—
Age of patients with vsCAP, *Me (Q1; Q3)*	68.0 (59.25; 78.0)	64.0 (57.0; 76.5)	71.0 (60.5; 80.0)	0.026 p_{23}=0.039
Length of stay in ICU, *Me (Q1; Q3)*	6.0 (3.0; 9.0)	5.0 (3.0; 9.0)	5.0 (2.0; 8.0)	0.283
Length of stay in the hospital, *Me (Q1; Q3)*)	15.0 (9.0; 21.0)	17.0 (12.0; 24.0)	25.0 (17.5; 32.0)	<0.001 p_{13}<0.001 p_{13}<0.001 p_{12}=0.002
Hospital mortality in patients with vsCAP, *N* (%)	106 (39.8)	76 (39.8)	54 (48.6)	0.239
Patients with bsCAP, *N* (%)	73 (76.0)	14 (14.6)	9 (9.4)	—
Age of patients with bsCAP, *Me (Q1; Q3)*	69.0 (62; 82.0)	70.5 (62.75; 80.5)	72.0 (66.0; 77.0)	0.955
Length of stay in ICU, *Me (Q1; Q3)*	7.0 (4.0; 13.3)	9.0 (7.0; 23.0)	7.0 (3.0; 10.0)	0.324
Length of stay in the hospital, *Me (Q1; Q3)*	7.5 (3.5; 17.0)	15.0 (11.0; 27.0)	29.0 (18.0; 32.0)	0.002 p_{13}=0.003
Hospital mortality in patients with bsCAP, *N* (%)	44 (60.3)	8 (57.1)	6 (66.7)	0.900

Table 4. Distribution in study groups according to APACHE IV scores.

APACHE IV score range	Frequency, *N* (%)			*P*
	vsCAP, *N*=568	bsCAP, *N*=96	Total, *N*=664	
41–52	160 (28.20)	11 (11.50)	171 (25.8)	0.0002
112–127	96 (16.90)	25 (26.00)	121 (18.2)	
53–60	73 (12.90)	8 (8.30)	81 (12.2)	
128–143	59 (10.40)	8 (8.30)	67 (10.1)	
79–92	43 (7.60)	10 (10.40)	53 (8.0)	
93–111	37 (6.50)	15 (15.60)	52 (7.8)	
144–195	34 (6.00)	11 (11.50)	45 (6.8)	
3–40	33 (5.80)	1 (1.00)	34 (5.1)	
61–68	17 (3.00)	5 (5.20)	22 (3.3)	
69–78	16 (2.80)	2 (2.10)	18 (2.7)	

A very strong correlation was found between actual adverse outcomes and the predicted mortality rate using the APACHE IV scale (η=0.966 for vsCAP and η=0.807 for bsCAP). The APACHE IV scale was used to predict ICU length of stay. The distribution of patients with sCAP across APACHE IV scores showed that 25.8% scored between 41–52 points. 18.2% scored between 112–127, 10.1% scored between 128–143 points, and 6.8% scored between 144–195 points (Table 4). Differences in APACHE IV score distribution between groups are shown in Table 4.

The median length of stay in the ICU for patients with vsCAP was 5 (3.0, 9.0) days compared to 7 (4.0, 14.0) days for patients with bsCAP. In the study by C. Dupuis et al., the median ICU stay for patients with bsCAP was 8.0 (4.0, 16.0) days [8]. According to an international report, the ICU length of stay for patients with vsCAP ranged from 5 to 19 days; our results are consistent with a British study [33]. Patients with bsCAP who scored 41–52 points had a significantly longer mean ICU length of stay than patients with vsCAP (*P*=0.001). The data of ICU length of stay for patients with vsCAP and bsCAP are shown in Table 5.

Patients with vsCAP whose APACHE IV scores ranged from 93 to 111 had ICU stays of 9 (5–12) or more days (Table 5). In the vsCAP group, the actual number of ICU days was significantly higher than predicted by APACHE IV scores in the 3–40 and 79–92 ranges, in contrast to the study by K. Zangmo et al. in which predicted days significantly exceeded actual days for patients with APACHE IV scores of 81–90 [15]. At a mean APACHE IV score of 99.92, actual and predicted ICU days were equivalent, a trend that persisted at higher mean scores (Table 6).

No significant correlation or concordance was found between predicted and actual ICU length of stay for patients with vsCAP (R^2=0.0257, Kendall's *W*=0.018). Fig. 2, *a* shows the correlation between actual and APACHE IV predicted ICU length of stay in the vsCAP.

In the bsCAP group, most patients (26.0%) had APACHE IV scores between 112 and 127, with a median ICU length of stay of 7.5 days (IQR 4.75–15.5). Ten patients (10.4%) with an APACHE IV score of 86.6 had an ICU length of stay of 10 days (IQR 6–13). In the bsCAP group, actual ICU length of stay was significantly longer than that predicted by APACHE IV scores in the 53–60 range, in contrast to the study by K. Zangmo et al. [15], which found that predicted ICU length of stay was significantly longer than actual ICU length of stay for APACHE IV scores of 50–60. No significant difference was found between predicted and actual ICU length of stay in bsCAP patients reaching a mean APACHE IV score of 64 (Table 7).

Table 5. Actual ICU length of stay in patients with vsCAP and bsCAP and different APACHE IV scores, *M±SD*; *Me (Q1–Q3)*.

APACHE IV		Mean ICU length of stay in groups		*P*
Score range	Mean score	vsCAP, *N*=568	bsCAP, *N*=96	
3–40	32.85	7.73±5.08 7 (4–10)	2.00	—
41–52	47.32	4.73±4.27 3 (2–6)	12.36±10.85 7 (5.5–14)	0.001
53–60	57.38	6.84±6.84 6 (3–8)	11.12±8.41 7.5 (7–11.75)	0.050
61–68	65.00	6.59±4.95 5 (4–7)	10.00±11.25 4 (2–16)	0.813
69–78	74.44	6.38±4.30 5 (4–8)	7.00±7.07 7 (4.5–9.5)	0.943
79–92	86.30	9.12±10.63 6 (4–9.5)	9.89±5.35 10 (6–13)	0.178
93–111	99.92	9.46±6.35 9 (5–12)	13.73±19.07 8 (5–11.5)	0.960
112–127	122.02	7.60±5.99 7 (3–10)	11.96±13.75 7.5 (4.75–15.5)	0.214
128–143	132.85	7.17±6.10 6 (3–9.5)	7.00±11.41 2 (1–5.75)	0.173
144–195	144.91	7.94±5.85 7.5 (4–10)	12.00±11.71 6 (4.5–16)	0.499

Table 6. Actual and predicted ICU length of stay in vsCAP patients with different APACHE IV scores, *M±SD*; *Me (Q1–Q3)*.

APACHE IV		vsCAP group, *N*=568	Mean ICU length of stay		*P*
Score range	Mean score	Number of patients, N (%)	Actual	Predicted	
3–40	32.88	33 (5.8)	7.73±5.08 7 (4–10)	3.34±0.86 3.3 (3.2–3.7)	<0.001
41–52	47.32	160 (28.1)	4.73±4.27 3 (2–6)	2.95±1.44 3.5 (1.2–4.2)	<0.001
53–60	57.38	73 (12.9)	6.84±6.84 6 (3–8)	4.44±0.71 4.2 (4.1–4.6)	<0.001
61–68	65.00	17 (3.0)	6.59±4.95 5 (4–7)	2.44±2.11 1.3 (1.0–3.3)	<0.001
69–78	74.44	16 (2.8)	6.38±4.30 5 (4–8)	3.39±2.07 4.1 (1.67–4.7)	0.016
79–92	86.30	43 (7.6)	9.12±10.63 6 (4–9.5)	5.48±2.44 4.8 (3.35–8)	0.043
93–111	99.92	37 (6.5)	9.46±6.35 9 (5–12)	7.22±1.01 7.5 (7.5–7.8)	0.101
112–127	122.02	96 (16.9)	7.60±5.99 7 (3–10)	7.62±0.47 7.65 (7.4–7.9)	0.220
128–143	132.85	59 (10.4)	7.17±6.10 6 (3–9.5)	6.88±0.51 6.9 (6.55–7.2)	0.435
144–195	144.91	34 (6.0)	7.94±5.85 7.5 (4–10)	6.13±0.37 6 (6.0–6.4)	0.096

Similar to the vsCAP group, we found no significant association or concordance between predicted and actual ICU length of stay in the bsCAP group (R^2=0.0294, Kendall's W=0.050). The correlation between actual and predicted ICU days using APACHE IV in the bsCAP group is shown in Fig. 2, *b*.

The accuracy of the APACHE IV model for predicting ICU length of stay > 1 and > 14 days was unsatisfactory for patients with vsCAP [AUROC 0.51 (95% CI: 0.441, 0.585) for > 1 day and 0. 595 (95% CI: 0.517, 0.674) for > 14 days] and for patients with bsCAP [AUROC 0.59 (95% CI: 0.379, 0.792) for > 1 day and 0.508 (95% CI: 0.371, 0.644) for > 14 days]. After adjustment for age ⩾ 60 years, the APACHE IV prediction model showed moderate performance in patients with vsCAP (AUROC 0.610).

In the bsCAP patient group, the use of the Charlson Comorbidity Index (CCI) yielded a predictive model of moderate quality (Table 8).

Predicting intensive care unit (ICU) bed occupancy is one of the most important tasks, as it enables planning and helps prevent overcrowding.

Accurate identification of high-risk groups, followed by appropriate patient routing to the ICU, is of paramount importance. An analysis of scoring systems revealed differences between patients with vsCAP and bsCAP according to major IDSA/ATS criteria, the NEWS2 scale (threshold ⩾ 2 points), and the REA-ICU scale (threshold ⩾ 7 points). In contrast, their scores on the SCAP scale (threshold ⩾ 10 points), PSI/PORT

Table 7. Actual and predicted ICU length of stay in bsCAP patients with different APACHE IV scores, *M±SD*; *Me (Q1–Q3)*.

APACHE IV		bsCAP group, *N*=96	Mean ICU length of stay		*P*
Score range	Mean score	Number of patients, *N* (%)	Actual	Predicted	
3–40	32.00	1 (1.0%)	2.00	5.50	—
41–52	47.36	11 (11.5%)	12.36±10.85 7 (5.5–14)	4.82±1.27 5.2 (5.2–5.2)	0.011
53–60	58.38	8 (8.3%)	11.12±8.41 7.5 (7–11.75)	4.85±2.68 6.3 (4.85–6.30)	0.014
61–68	64.00	5 (5.2%)	10.00±11.25 4 (2–16)	5.66±2.83 7.2 (5.5–7.4)	0.625
69–78	70.50	2 (2.1%)	7.00±7.07 7 (4.5–9.5)	4.40±0.85 4.4 (4.1–4.7)	1.000
79–92	86.60	10 (10.4%)	9.89±5.35 10 (6–13)	7.07±2.40 8.1 (8–8.28)	0.129
93–111	97.47	15 (15.6%)	13.73±19.07 8 (5–11.5)	7.05±1.22 7.5 (7.45–7.70)	0.410
112–127	120.32	25 (26.0%)	11.96±13.75 7.5 (4.75–15.5)	7.15±1.03 7.5 (7.3–7.8)	0.141
128–143	132.00	8 (8.3%)	7.00±11.41 2 (1–5.75)	6.20±0.84 6.5 (5.4–6.85)	0.622
144–195	147.73	11 (11.5%)	12.00±11.71 6 (4.5–16)	5.65±0.93 6 (5.15–6.30)	0.262

(> 130 points), SMART-COP (⩾ 3 points), CURB-65 (⩾ 3 points), and minor IDSA/ATS criteria did not show significant differences.

It was found that 73 (76.0%) patients with bsCAP and 266 (46.8%) patients with vsCAP were admitted to the ICU within < 48 hours. A delay in ICU admission of more than 7 days was observed in older patients with severe CAP. This delay was associated with longer hospital stay (*P*<0.0001), but did not have a significant impact on ICU length of stay or patient outcomes.

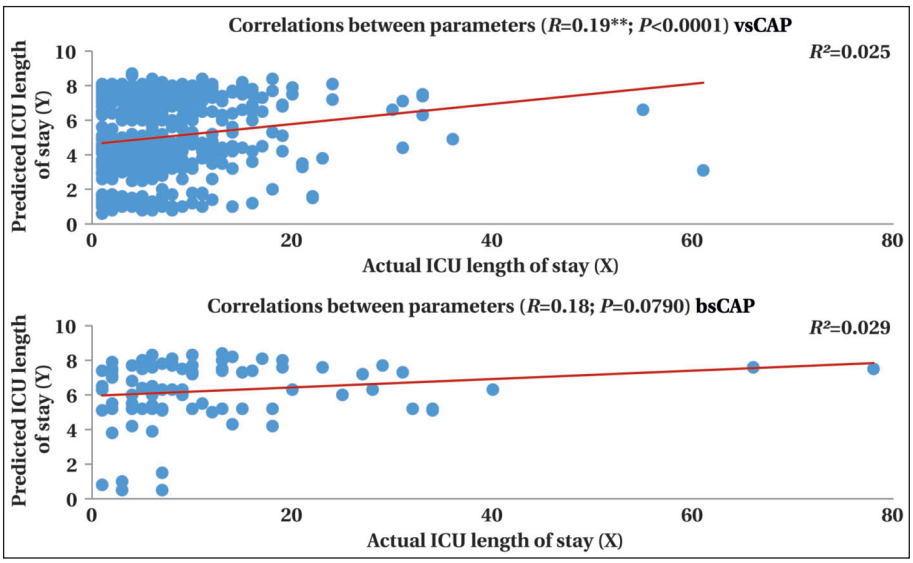

Fig. 2. Correlation between actual and predicted ICU length of stay based on APACHE IV in vsCAP *(a)* and bsCAP *(b)* groups.

Notably, 37 patients (6.5%) with vsCAP and APACHE IV scores of 93–111 and 10 patients (10.4%) with bsCAP and APACHE IV scores of 79–92 required prolonged ICU hospitalization.

A very strong correlation was found between actual adverse outcomes and predicted mortality according to the APACHE IV scale. However, no significant association and concordance was found

Table 8. Prediction of prolonged ICU stay (> 14 days) in patients (*N*=81).

Scale	Values in different groups							*P*
	Threshold	AUROC 95% CI	Se/Sp,%	*P*	Threshold	AUROC 95% CI	Se/Sp,%	
vsCAP								
	All patients with vsCAP (*N*=57)				Patients with vsCAP ⩾60 years old (*N*=44)			
APACHE IV	⩾65.0	0.595 [0.517; 0.674]	71.93/49.71	0.002	⩾66.0	0.610 [0.528; 0.692]	84.09/43.82	<0.001
CFS	⩾4.0	0.575 [0.496; 0.653]	52.63/63.46	0.018	⩾4.0	0.579 [0.492; 0.665]	65.91/50.27	0.042
CCI	⩾4.0	0.544 [0.467; 0.620]	59.65/53.62	0.057	⩾4.0	0.529 [0.444; 0.614]	68.18/44.62	0.105
bsCAP								
	All patients with bsCAP (*N*=24)				Patients with bsCAP ⩾60 years old (*N*=22)			
APACHE IV	⩾115.0	0.508 [0.371; 0.644]	45.83/64.29	0.379	⩾115.0	0.517 [0.373; 0.661]	45.45/64.29	0.426
CFS	⩾7.0	0.625 [0.452; 0.797]	54.55/67.44	0.178	⩾7.0	0.539 [0.331; 0.746]	60.00/54.84	0.414
CCI	⩾7.0	0.615 [0.481; 0.749]	79.17/44.93	0.037	⩾7.0	0.586 [0.441; 0.732]	81.82/34.55	0.156

Note. AUC — area under the ROC curve; Se — sensitivity; Sp — specificity; CCI — Charlson Comorbidity Index; CFS — Clinical Frailty Scale.

between actual and predicted ICU length of stay in patients with severe CAP, which may be due to the specific characteristics of the ICU and the hospital, as well as the severity of the patients' conditions at the time of ICU admission.

Elderly and senile patients predominated in both groups. Respiratory support of various modalities was required in 567 patients (99.8%) with vsCAP and 80 patients (83.3%) with bsCAP ($P<0.001$). In addition, 203 patients (35.7%) with vsCAP had a bacterial co-infection on admission to the ICU. Patients with bsCAP had higher Charlson Comorbidity Index (CCI) scores, while their Clinical Frailty Scale (CFS) scores indicated a need for personalized care due to significant physical and cognitive impairment.

Age-adjusted analysis of the predictive model, including only patients older than 60 years, showed moderate predictive accuracy of ICU length of stay for the APACHE IV scale in patients with vsCAP. However, in the bsCAP group, the APACHE IV scale showed poor predictive performance. In this group, moderate predictive accuracy was achieved using the Charlson Comorbidity Index (CCI).

Study limitation. Data were obtained from a single-center study.

Conclusion

Significant differences were found in the NEWS2, REA-ICU, and major IDSA/ATS criteria for ICU routing of patients with bacterial and viral severe community-acquired pneumonia.

The APACHE IV scale showed a very strong correlation between predicted and actual mortality rates and no correlation between predicted and actual ICU length of stay for patients with severe community-acquired pneumonia.

Appendix

Table of scales used in the study of patients with sCAP.

№	Scale	Description
		Severity Assessment
1.	SMART-COP/SMRT-CO (systolic blood pressure, multilobar infiltration, albumin, respiratory rate, tachycardia, confusion, oxygenation, pH), 2008 [18].	Australian model for identifying patients needing respiratory support and catecholamine infusion based on 8 clinical parameters.
2.	PSI/PORT (Pneumonia Severity Index — Pneumonia Patient Outcomes Research Team), 1997 [19].	A two-step scoring system based on demographic, clinical, laboratory, and radiological parameters. Patients are classified into one of five classes (I–V), which guide routing and mortality prediction.
3.	REA-ICU (Risk of Early Admission to the ICU) 2009 [20].	A mixed French-American risk assessment for early ICU admission.
4.	CURB-65 (confusion, uremia, respiratory rate, blood pressure, age ⩾65 years), 2003 [21].	Proposed by the British Thoracic Society to assess the severity of community-acquired pneumonia and guide patient routing.
5.	IDSA/ATS (American Thoracic Society Criteria for Defining Severe Community-acquired Pneumonia) 2007 [22].	American Thoracic Society and Infectious Diseases Society model, consisting of major and minor criteria based on the need for respiratory and vasopressor support, as well as clinical, radiological, and laboratory parameters.
6.	SCAP (Severe Community-Acquired Pneumonia score) 2009 [23].	Spanish model used to predict 30-day mortality based on 8 clinical, laboratory, and radiological parameters.
7.	NEWS 2 (National Early Warning Score), 2017 [24].	British standardized patient severity assessment based on 7 clinical parameters.
8.	SOFA (Sequential Organ Failure Assessment) 1996 [25].	Organ dysfunction assessed based on 6 organ systems every 24 hours from admission to transfer.
		Duration of ICU stay
9.	APACHE IV (Acute Physiology and Chronic Health Evaluation IV) 2006 [26].	APACHE IV model used for predicting mortality and ICU stay duration.
10.	CFS (The Clinical Frailty Scale) [27].	A frailty assessment tool based on judgment, evaluating comorbidities, performance, and cognitive status, providing a frailty score from 1 (very fit) to 9 (terminally ill).
11.	CCI (Charlson Comorbidity Index) [28].	Index predicting 10-year survival based on age and comorbidities.
12.	MPM (Mortality Probability Model 0–III) 2007 г. [13]	A scale for predicting mortality.
13.	SAPS II (new Simplified Acute Physiology	A scale for assessing ICU severity and predicting mortality based on 17 variables: 12 clinical-laboratory parameters, age, type of hospitalization (elective surgery, emergency surgery, or medical), and three variables of primary disease (AIDS, metastatic cancer, and hematological malignancies).

References

1. *Song Y., Wang X., Lang K., Wei T., Luo J., Song Y., Yang D.* Development and validation of a nomogram for predicting 28-day mortality on admission in elderly patients with severe community-acquired pneumonia. *J Inflamm Res.* 2022; 15: 4149–4158. DOI: 10.2147/JIR.S369319. PMID: 35903289.
2. *Авдеев С. Н., Белобородов В. Б., Белоцерковский Б. З., Грицан А. И., Дехнич А. В., Зайцев А. А., Киров М. Ю., и др.* Тяжелая внебольничная пневмония у взрослых. Клинические рекомендации Федерации анестезиологов и реаниматологов России. *Анестезиология и реаниматология.* 2022; (1): 6–35. *Avdeev S. N., Beloborodov V. B., Belotserkovskiy B. Z., Gritsan A. I., Dekhnich A. V., Zaitsev A. A., Kirov M. Yu., et al.* Severe community-acquired pneumonia in adults. Clinical recommendations from Russian Federation of Anaesthesiologists and Reanimaologists. *Russian Journal of Anesthesiology and Reanimatology = Anesteziologiya i Reanimatologiya.* 2022; (1): 6–35. (In Russ.). DOI: 10.17116/anaesthesiology20220116
3. *Church S., Rogers E., Rockwood K., Theou O.* A scoping review of the Clinical Frailty Scale. *BMC Geriatr.* 2020; 20 (1): 393. DOI: 10.1186/s12877-020-01801-7. PMID: 33028215
4. *Martin-Loeches I., Torres A.* Severe Community-Acquired Pneumonia. *Semin Respir Crit Care Med.* 2024; 45 (2): 141–142. DOI: 10.1055/s-0044-1780515. PMID: 38604187
5. *Marti C., Garin N., Grosgurin O., Poncet A., Combescure C., Carballo S., Perrier A.* Prediction of severe community-acquired pneumonia: a systematic review and meta-analysis. *Crit Care.* 2012; 16 (4): R141. DOI: 10.1186/cc11447. PMID: 22839689.
6. *Fukuyama H., Ishida T., Tachibana H., Nakagawa H., Iwasaku M., Saigusa M., Yoshioka H., et al.* Validation of scoring systems for predicting severe community-acquired pneumonia. *Intern Med.* 2011; 50 (18): 1917–1922. DOI: 10.2169/internalmedicine.50.5279. PMID: 21921369.
7. *Divino V., Schranz J., Early M., Shah H., Jiang M., DeKoven M.* The annual economic burden among patients hospitalized for community-acquired pneumonia (CAP): a retrospective US cohort study. *Curr Med Res Opin.* 2020; 36 (1): 151–160. DOI: 10.1080/03007995.2019.1675149. PMID: 31566005.
8. *Dupuis C., Sabra A., Patrier J., Chaize G., Saighi A., Feger C., Vainchtock A., et al.* Burden of pneumococcal pneumonia requiring ICU admission in France: 1-year prognosis, resources use, and costs. *Crit Care.* 2021; 25 (1): 24. DOI: 10.1186/s13054-020-03442-z. PMID: 33423691.
9. *Çelikhisar H., Daşdemir Ilkhan G., Arabaci Ç.* Prognostic factors in elderly patients admitted to the intensive care unit with community-acquired pneumonia. *Aging Male.* 2020; 23 (5): 1425–1431. DOI: 10.1080/13685538.2020.1775192. PMID: 32543939.
10. *Haessler S., Guo N., Deshpande A., Zilberberg M. D., Lagu T., Lindenauer P. K., Imrey P. B., et al.* Etiology, treatments, and outcomes of patients with severe community-acquired pneumonia in a large U.S. sample. *Crit Care Med.* 2022; 50 (7): 1063–1071. DOI: 10.1097/CCM.0000000000005498. PMID: 35191410.
11. *Woods A. W., MacKirdy F. N., Livingston B. M., Norrie J., Howie J. C.* Evaluation of predicted and actual length of stay in 22 Scottish intensive care units using the APACHE III system. Acute Physiology and Chronic Health Evaluation. *Anaesthesia.* 2000; 55 (11): 1058–1065. DOI: 10.1046/j.1365-2044.2000.01552.x. PMID: 11069331
12. *Vasilevskis E. E., Kuzniewicz M. W., Cason B. A., Lane R. K., Dean M. L., Clay T., Rennie D. J., et al.* Mortality probability model III and simplified acute physiology score II: assessing their value in predicting length of stay and comparison to APACHE IV. *Chest.* 2009; 136 (1): 89–101. DOI: 10.1378/chest.08-2591. PMID: 19363210.
13. *Higgins T. L., Teres D., Copes W. S., Nathanson B. H., Stark M., Kramer A. A.* Assessing contemporary intensive care unit outcome: an updated Mortality Probability Admission Model (MPM0-III). *Crit Care Med.* 2007; 35 (3): 827–835. DOI: 10.1097/01.CCM.0000257337.63529.9F. PMID: 17255863.
14. *Le Gall J. R., Lemeshow S., Saulnier F.* A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study [published correction appears in JAMA 1994 May 4; 271 (17): 1321]. *JAMA.* 1993; 270 (24): 2957–2963. DOI: 10.1001/jama.270.24.2957. PMID: 8254858.
15. *Zangmo K., Khwannimit B.* Validating the APACHE IV score in predicting length of stay in the intensive care unit among patients with sepsis. *Sci Rep.* 2023; 13 (1): 5899. DOI: 10.1038/s41598-023-33173-4. PMID: 37041277.

16. *Takekawa D., Endo H., Hashiba E., Hirota K.* Predict models for prolonged ICU stay using APACHE II, APACHE III and SAPS II scores: A Japanese multicenter retrospective cohort study. *PLoS One.* 2022; 17 (6): e0269737. DOI: 10.1371/journal.pone.0269737. PMID: 35709080.
17. *Bahlis L. F., Diogo L. P., Fuchs S. C.* Charlson Comorbidity Index and other predictors of in-hospital mortality among adults with community-acquired pneumonia. *J Bras Pneumol.* 2021; 47 (1): e20200257. DOI: 10.36416/1806-3756/e20200257. PMID: 33656092.
18. *Charles P. G., Wolfe R., Whitby M., Fine M. J., Fuller A. J., Stirling R., Wright A. A., et al.* SMART-COP: a tool for predicting the need for intensive respiratory or vasopressor support in community-acquired pneumonia. *Clin Infect Dis.* 2008; 47 (3): 375–84. DOI: 10.1086/589754. PMID: 18558884.
19. *Fine M. J., Auble T. E., Yealy D. M., Hanusa B. H., Weissfeld L. A., Singer D. E., Coley C. M., et al.* A prediction rule to identify low-risk patients with community-acquired pneumonia. *N Engl J Med.* 1997; 336 (4): 243–250. DOI: 10.1056/NEJM199701233360402. PMID: 8995086.
20. *Renaud B., Labarère J., Coma E., Santin A., Hayon J., Gurgui M., Camus N., et al.* Risk stratification of early admission to the intensive care unit of patients with no major criteria of severe community-acquired pneumonia: development of an international prediction rule. *Crit Care.* 2009; 13 (2): R54. DOI: 10.1186/cc7781. PMID: 19358736.
21. *Liu J.-L., Xu F., Zhou H., Wu X.-J., Shi L.-X., Lu R.-Q., Farcomeni A., Farcomeni A., Venditti M., et al.* Expanded CURB-65: a new score system predicts severity of community-acquired pneumonia with superior efficiency. *Sci Rep.* 2016; 6: 22911. DOI: 10.1038/srep22911. PMID: 26987602.
22. *Mandell L. A., Wunderink R. G., Anzueto A., Bartlett J. G., Campbell G. D., Dean N. C., Dowell S. F., et al.* Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis.* 2007; 44 (Suppl 2): S27–72. DOI: 10.1086/511159. PMID: 17278083.
23. *España P. P., Capelastegui A., Quintana J. M., Bilbao A., Diez R., Pascual S., Esteban C., et al.* Validation and comparison of SCAP as a predictive score for identifying low-risk patients in community-acquired pneumonia. *J Infect.* 2010; 60 (2): 106–113. DOI: 10.1016/j.jinf.2009.11.013. PMID: 19961875.
24. National Early Warning Score (NEWS) 2 — Standardising the assessment of acute illness severity in the NHS. ISBN 978-1-86016-682-2.588210.
25. *Zimmerman J. E., Kramer A. A., McNair D. S., Malila F. M., Shaffer V. L.* Intensive care unit length of stay: benchmarking based on Acute Physiology and Chronic Health Evaluation (APACHE) IV. *Crit Care Med.* 2006; 34 (10): 2517–2529. DOI: 10.1097/01.CCM.0000240233.01711.D9. PMID: 16932234
26. *Sternberg S. A., Wershof Schwartz A., Karunananthan S., Bergman H., Mark Clarfield A.* The identification of frailty: a systematic literature review. *J Am Geriatr Soc.* 2011; 59 (11): 2129–2138. DOI: 10.1111/j.1532-5415.2011.03597.x. PMID: 22091630.
27. *Charlson M. E., Pompei P., Ales K. L., MacKenzie C. R.* A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis.* 1987; 40 (5): 373–383. DOI: 10.1016/0021-9681(87)90171-8. PMID: 3558716.
28. *Covino M., Sandroni C., Della Polla D., De Matteis G., Piccioni A., De Vita A., Russo A., et al.* Predicting ICU admission and death in the Emergency Department: a comparison of six early warning scores. *Resuscitation.* 2023; 190: 109876. DOI: 10.1016/j.resuscitation.2023.109876. PMID: 37331563.
29. *Neto F. L., Marino L. O., Torres A., Cilloniz C., Marchini J. F. M., de Alencar J. C. G., Palomeque A., et al.* Community-acquired pneumonia severity assessment tools in patients hospitalized with COVID-19: a validation and clinical applicability study. *Clin Microbiol Infect.* 2021; 27 (7): 1037.e1–1037.e8. DOI: 10.1016/j.cmi.2021.03.002. PMID: 33813111.
30. *Liapikou A., Ferrer M., Polverino E., Balasso V., Esperatti M., Piner R., Mensa J., et al.* Severe community-acquired pneumonia: validation of the Infectious Diseases Society of America/American Thoracic Society guidelines to predict an intensive care unit admission. *Clin Infect Dis.* 2009; 48 (4): 377–385. DOI: 10.1086/596307. PMID: 19140759.
31. *Woodhead M., Welch C. A., Harrison D. A., Bellingan G., Ayres J. G.* Community-acquired pneumonia on the intensive care unit: secondary analysis of 17,869 cases in the ICNARC Case

Mix Programme Database. *Crit Care.* 2006; 10 (Suppl 2): S1.
DOI: 10.1186/cc4927. PMID: 16934135.

32. *Rees E. M., Nightingale E. S., Jafari Y., Waterlow N. R., Clifford S., Pearson C. A. B., Jombart T., et al.* COVID-19 length of hospital stay: a systematic review and data synthesis. *BMC Med.* 2020; 18 (1): 270.
DOI: 10.1186/s12916-020-01726-3.
PMID: 32878619.

Received 02.04.2024
Accepted 05.11.2024

https://doi.org/10.15360/1813-9779-2025-1-49-54

Prolonged Inhalation Sedation in the Intensive Care Unit Using the AnaConDa Device (Case Reports)

Alexey P. Achkasov[1,2]*, Rostislav A. Cherpakov[1,2]*, Shamil Zh. Khusainov[1,2], Vladimir V. Kulabukhov[2], Petr A. Yartsev[2], Dmitry I. Levikov[3], Sergey N. Kuznetsov[2], Aslan K. Shabanov[1,2]

[1] Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[2] N. V. Sklifosovsky Research Institute of Emergency Medicine, Moscow City Health Department, 3 Bolshaya Sukharevskaya Square, Bldg. 1, 129090 Moscow, Russia
[3] S. P. Botkin City Clinical Hospital, 5 Botkin 2nd Ave, 125284 Moscow, Russia

For citation: *Alexey P. Achkasov, Rostislav A. Cherpakov, Shamil Zh. Khusainov, Vladimir V. Kulabukhov, Petr A. Yartsev, Dmitry I. Levikov, Sergey N. Kuznetsov, Aslan K. Shabanov.* Prolonged Inhalation Sedation in the Intensive Care Unit Using the AnaConDa Device (Case Reports). *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 49–54. https://doi.org/10.15360/1813-9779-2025-1-49-54 [In Russ. and Engl.]

***Correspondence to:** Alexey P. Achkasov, alexachkas@gmail.com; Rostislav A. Cherpakov, rcherpakov@fnkcrr.ru

Summary

This report describes two clinical cases involving prolonged inhalation sedation using the AnaConDa device in the ICU. Both patients achieved and maintained adequate sedation levels throughout the treatment period. No significant adverse cardiovascular effects were observed.

Keywords: inhalation sedation; AnaConDa; prolonged sedation; sepsis

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

Information about the authors:
Alexey P. Achkasov: ORCID 0009-0008-3299-6005
Rostislav A. Cherpakov: ORCID 0000-0002-0514-2177
Shamil Zh. Khusainov: ORCID 0000-0002-3177-8929
Vladimir V. Kulabukhov: ORCID 0000-0003-1769-7038
Petr A. Yartsev: ORCID 0000-0003-1270-5414
Dmitry I. Levikov: ORCID 0000-0002-3614-6971
Sergey N. Kuznetso: ORCID 0000-0002-7881-5336
Aslan K. Shabanov: ORCID 0000-0002-3417-2682

Introduction

The challenge of sedation in the intensive care unit (ICU) remains a pressing issue despite the availability of several intravenous sedative agents [1, 2]. Each of these agents has potential drawbacks that may adversely affect patient outcomes. Nevertheless, a growing body of evidence, although still limited, supports the routine use of the AnaConDa device for inhaled sedation [3, 4]. The organ-protective properties of sevoflurane and the absence of reported tachyphylaxis suggest its potential efficacy in the ICU setting [5].

Case Report No.1

A 26-year-old man with a height of 186 cm and a weight of 77 kg (BMI 22.3 kg/m²) was admitted to the ICU with altered consciousness following substance use.

Emergency medical services (EMS) reported that the patient had experienced a generalized seizure prior to arrival at the hospital, which was effectively treated with 10 mg of diazepam. Due to the ambiguous clinical presentation of intoxication, no antidotal therapy was administered.

Urine toxicology revealed cannabinoids, cocaine, methadone and its metabolite (2-ethylidene-1.5-dimethyl-3.3-diphenylpyrrolidine), and alpha-PVP.

On admission, the patient had a Glasgow Coma Scale (GCS) score of 12, indicating severe stupor, with episodes of psychomotor agitation and central tachypnea due to intoxication syndrome, with a respiratory rate (RR) of 30 breaths per minute.

CT scan of the brain: Cystic gliotic lesions in the right frontal lobe.

Chest CT scan: Inflammatory changes in the lower lobe segments of the right lung, consistent with aspiration pneumonia secondary to decreased consciousness.

Laboratory tests showed significant abnormalities:

- Hemoconcentration: Hematocrit 49%, hemoglobin 163 g/dL.
- Mixed acidosis (arterial blood gas):
  - pH 6.97
  - PO_2 125 mmHg,
  - P/F ratio 357 mmHg
  - PCO_2 85 mmHg
  - HCO_3 19.6 mmol/L

- Base Excess (BE) –16.1 mmol/L
- Lactate 7.3 mmol/L

- Hyperglycemia: glucose 20.5 mmol/L.
- Systemic inflammatory markers:
  - Leukocytes 18.42 × 10^9/L
  - C-reactive protein (CRP) 62.2 mg/L
  - Procalcitonin (PCT) 3.5 ng/mL.

Due to marked psychomotor agitation, impaired consciousness, and tachypnea associated with the intoxication syndrome, the patient required pharmacologic sedation to prevent cerebral complications. Endotracheal intubation was performed, and the patient was placed on mechanical ventilation (SIMV mode) with the following parameters:

- Tidal volume (V_t) 500 mL
- Inspiratory time (T_{insp}) 1.35 sec
- Respiration frequency (F) 16/min
- Positive end-expiratory pressure (PEEP) 8 cm H_2O
- Fraction of inspired oxygen (FiO_2) 40%
- Assisted spontaneous breathing pressure (P_{asb}) 16 cm H_2O

With these settings, the monitoring parameters were as follows:

- Peripheral oxygen saturation (SpO_2) 99%.
- Minute ventilation (MV) 8 L/min
- End tidal CO_2 ($EtCO_2$) 37 mmHg.

Intravenous sedation with propofol was initiated at an initial dose of 2 mg/kg/h, achieving a Richmond Agitation-Sedation Scale (RASS) score of –4.

Despite the aggressive therapy, the patient showed hemodynamic instability requiring vasopressor and inotropic support with norepinephrine (initial dose 0.6 µg/kg/min) and epinephrine (0.4 µg/kg/min), with a calculated vasopressor-inotropic score (VIS) of 100 points [6]. Continuous invasive blood pressure monitoring was started.

The patient was started on antimicrobial therapy with ampicillin-sulbactam (3 g three times daily), fluid resuscitation (40 mL/kg/day), gastroprotective therapy with omeprazole (40 mg twice daily), and anticoagulation with nadroparin (2.850 IU anti-Xa twice daily).

With correction of blood gas and metabolic parameters, the following improvements were observed:

- pH 7.470
- PCO_2 36.9 mmHg
- P/F ratio 312 mmHg
- HCO_3 26.5 mmol/L
- BE 3.4 mmol/L
- Glucose 6.3 mmol/L
- Lactate 2.4 mmol/L

Consciousness fully recovered; however, as sedation was tapered, the patient developed severe anxiety, psychomotor agitation, tachycardia, and ventilator asynchrony. Because of these complications, continued mechanical ventilation was deemed necessary.

To achieve adequate depth of sedation, propofol was increased to > 4 mg/kg/h [5]. Due to this high requirement, inhaled sedation with sevoflurane was initiated using the AnaConDa device at a rate of 5 mL/h, adjusted based on gas analyzer readings to maintain a target minimum alveolar concentration (MAC) of 0.5–1.0% vol.

The patient remained on SIMV mode ventilation with the following parameters:

- V_t 550 mL
- T_{insp} 1.4 sec
- F 16/min
- PEEP 6 cm H_2O
- FiO_2 40%
- P_{asb} 12 cm H_2O

With these settings, the monitoring parameters were as follows

- SpO_2 96%.
- MV 8.8 L/min
- $EtCO_2$ 40 mmHg.

During the first 12 hours, the patient remained hemodynamically unstable and dependent on sympathomimetic support. However, within 24 hours of initiation of inhaled sedation, the signs of acute cardiovascular failure subsided and vasopressor therapy was discontinued. The patient regained full consciousness.

With normalization of acid-base balance and arterial blood gas parameters (pH 7.37, PCO_2 37.3 mmHg, P/F ratio 353 mmHg, HCO_3 21.1 mmol/L, BE 3.1 mmol/L, glucose 6.8 mmol/L, lactate 1.9 mmol/L), along with restored alertness, adequate muscle tone, and spontaneous breathing, the patient was successfully extubated (Table 1).

Six hours after extubation, the patient was transferred to the appropriate clinical unit and discharged from the hospital the following day after refusing further treatment.

Case Report No.2

A 43-year-old female patient (height 170 cm, weight 68 kg) was admitted to the ICU with a clinical presentation of septic shock (hypotension with BP 70/40 mmHg, HR 125/min, severe tachypnea) due to obstructive pyelonephritis. Laboratory findings showed signs of systemic inflammation (leucocyte count 20.24 × 10^9/L, platelet count 78 × 10^9/L, CRP 229.4 mg/L, PCT 10.6 ng/mL) and metabolic acidosis (pH 7.29, BE -9.1 mmol/L, SvO_2 53%, lactate 7.1 mmol/L).

Emergency right ureteral stenting was performed and comprehensive intensive therapy was initiated, including vasopressor support (norepinephrine at 2.3 µg/kg/min, VIS score 230), fluid resuscitation (30 mL/kg), antimicrobial therapy (cefepime+sulbactam 4 g twice daily + metronidazole 500 mg three times daily + fosfomycin 4 g three times daily), and noninvasive lung ventilation (NILV).

Table 1. Changes in instrumental and laboratory data of patient No. 1.

Parameter	Values at different study steps					
	prior to MLV	MLV, sedation				after MLV (extubation)
		Intravenous (propofol)		Inhalation (sevoflurane)		
		12 h	24 h	12 h	24 h	
P/F ratio, mm Hg	357	377	342	362	451	353
MAP, mm Hg	87	51	79	68	104	92
Norepinephrine, μg/kg/min	—	0.6	0.6	0.6	0.25	—
Epinephrine, μg/kg/min	—	0.4	0.1	0.08	—	—
pH(a)	6.97	7.13	7.34	7.47	7.38	7.37
PCO_2, mm Hg	85	76	47	36	34	37
Lactate, mmol/L	7.3	1.3	1.6	2.4	1.7	1.9
PEEP, cm H_2O	—	8	7	6	6	—
EF, %	62	62	62	62	62	62

Note. For Tables 1–3: MLV — mechanical lung ventilation; MAP — mean arterial pressure; pH(a) — arterial blood pH; PEEP — positive end-expiratory pressure; EF — ejection fraction.

However, the patient's condition continued to deteriorate: persistent hypotension refractory to sympathomimetics, tachypnea of central origin, and episodes of confusion alternating with psychomotor agitation were observed. These symptoms were interpreted as manifestations of hypoxic encephalopathy.

It was decided to initiate mechanical ventilation. The patient underwent endotracheal intubation and mechanical ventilation was performed with a Drager XL ventilator in SIMV mode: V_t 480 mL, T_{insp} 1.4 seconds, F 15 breaths/minute, PEEP 8 cm H_2O, FiO_2 60%, and Pasb 16 cm H_2O. With these settings, monitoring parameters were as follows: SpO_2 99%, MV 7.2 L/min, and $EtCO_2$ 32 mmHg. Given these parameters, sedation was necessary. Initial arterial blood gas analysis at the start of mechanical ventilation showed: pH 7.209, PaO_2 108 mmHg, $PaCO_2$ 37 mmHg, bicarbonate (HCO_3) 14.4 mmol/L, base excess (BE) –13.5 mmol/L, and lactate 4.0 mmol/L.

Due to persistent hypotension, invasive hemodynamic monitoring was performed using the PICCO method with the following parameters: cardiac index (CI) 3.34 L/min/m², stroke volume index (SVI) 28 mL/m², systemic vascular resistance index (SVRI) 1852 dyn×s×cm^{-5}×m², cardiac power index (CPI) 0.66 W/m², global end-diastolic volume (GEDV) 686 mL/m², extravascular lung water index (ELWI) 14 mL/kg, global ejection fraction (GEF) 16%, and cardiac function index (CFI) 5.2 min^{-1}. Inotropic support was initiated with dobutamine at 5 μg/kg/min.

Due to the presence of acute kidney injury with preserved urine output and lactate acidosis, a combined extracorporeal therapy approach was used within the first 24 hours of hospitalization. This included hemodiafiltration with an EMIC2 filter and hemoadsorption with the Cytosorb system.

To maintain adequate sedation, a multidrug protocol was required that included high doses of ketamine, midazolam, fentanyl, and dexmedetomidine (Dexdor). The dosing regimen included dexmedetomidine at 0.8 μg/kg/h, ketamine at 150 mg/h, midazolam at 7.5 mg/h, and fentanyl at 0.2 mg/h. Based on these requirements, inhalation sedation with sevoflurane was initiated using the AnaConDa system.

Sevoflurane sedation was monitored by gas analyzer readings and continued for a total of 72 hours (Table 2). During this time, extracorporeal therapy was discontinued and both vasopressor and inotropic support were gradually tapered. The patient's VIS score decreased over three days from 230 to 87 and finally to 30. Laboratory tests showed normalization of acid-base balance and blood gas levels (Table 3), and signs of systemic inflammation resolved.

Table 2. Central hemodynamic parameters of patient No. 2.

Parameters	Values during sedation				
	Intravenous	Inhalation (Sevoflurane)			
		12 h	24 h	48 h	72 h
MAP, mm Hg	85	92	94	89	85
HR, bpm	120	110	99	107	95
Norepinephrine, μg/kg/min	1.4	0.87	0.5	0.3	0.15
Dobutamine, μg/kg/min	5	3.57	2.0	1.19	2.0
CI, L/min/m²	3.34	3.93	3.86	3.69	6.04
SVRI, dyn×s×cm^{-5}×m²	1852	1766	1696	2043	952
ELWI, mL/kg	14	16	16	16	14
GEF, %	16	18	19	23	27
GEDV, mL/m²	686	810	869	790	870
PEEP, cm H_2O	8	10	9	8	8

Note. HR — heart rate; CI — cardiac index; SVRI — systemic vascular resistance index; ELWI — extravascular lung water index; GEF — global ejection fraction; GEDV — global end-diastolic volume.

Table 3. Changes in instrumental and laboratory parameters of patient No. 2.

Parameter	Values during study steps					
	MLV, sedation					after MLV (extubation)
	Intravenous	Inhalation (Sevoflurane)				
		12 h	24 h	48 h	72 h	
P/F ratio, mm Hg	262	330	285	255	332	383
pH (a)	7.43	7.47	7.49	7.42	7.48	7.54
PCO_2, mm Hg	40	37	38	42	40	34
Lactate, mmol/L	3.9	2.0	2.34	2.5	0.85	1.23
BE, mmol/L	2.1	3.2	0.4	2.5	5.6	4.3
HCO_3, mmol/L	26.5	26.9	24.7	27.1	29.5	27.3
SvO_2, %	87	86	87	85	88	89
Leucocytes, 10^9/L	16.3	13.6	9.3	13.4	16.2	11.5
Platelets, 10^9/L	55	43	23	77	60	64

The patient's level of consciousness outside of sedation was assessed daily using the FOUR score. The score was 12 on the first day, 14 on the second day, and 16 on the third day. When the patient regained full consciousness, full muscle tone, and adequate spontaneous respiration, she was successfully extubated.

Subsequent rehabilitation in the intensive care unit was uneventful. After three days, the patient was transferred to a specialized unit and later discharged from the hospital.

Discussion

Despite advances in surgical techniques for the treatment of patients with acute abdominal pathology [7], the availability of a broad spectrum of antibacterial agents [8], the development of extracorporeal therapies [9], and improvements in early diagnosis [10], sepsis remains a critical problem in modern clinical practice [11]. The high mortality, complexity and prolonged duration of treatment, including prolonged stays in intensive care units, necessitate a continuous search for new strategies to mitigate the consequences of sepsis and septic shock.

A particular challenge is that patients with septic shock often require prolonged mechanical ventilation. This in turn is associated with several secondary complications, including ventilator-associated pneumonia, the need for prolonged sedation, and subsequent difficulties in early patient mobilization.

Traditional sedatives in the ICU include propofol and midazolam [12–14]. However, prolonged use of these drugs is associated with several challenges that may further complicate the already complex prognosis in this patient population.

A major concern is propofol infusion syndrome [15, 16]. Continuous administration at doses greater than 4 mg/kg/h for more than 72 hours can alter mitochondrial respiratory chain function, leading to impaired oxidative phosphorylation and accumulation of anaerobic metabolic by-products [15].

Midazolam, on the other hand, is associated with rapid development of tolerance [17]. In cases requiring prolonged sedation, this may result in reaching the maximum tolerated dose relatively quickly without achieving adequate sedation, even in combination with opioid analgesics.

Dexmedetomidine, a selective α_2-adrenergic receptor agonist, has become an integral part of clinical practice, demonstrating efficacy as a sedative in a wide range of conditions. Recent experimental studies [18] suggest that dexmedetomidine may also have organ-protective effects on the lungs in septic shock. In addition, several studies since 2010 [19] and continuing to the present [20, 21] have reported a significant reduction in vasopressor requirements in patients who received dexmedetomidine sedation from the onset of illness. However, similar to midazolam, prolonged use of dexmedetomidine is associated with the development of tachyphylaxis.

For a long time, inhalation sedation was not widely used in the ICU due to technical challenges. However, in 2012 (and in Europe since the early 2000s), the AnaConDa (The Anaesthetic Conserving Device) was certified in Russia, allowing the safe and effective use of inhalational anesthetics in critical care. This advancement allowed clinical application of the previously established organ-protective properties of sevoflurane [21–25] in patients with septic shock, with the goal of reducing multiple organ dysfunction.

Concerns regarding the hemodynamic effects of sevoflurane in patients with circulatory failure due to septic shock have not been substantiated by numerous studies [22, 23, 26]. The only retrospective study evaluating the effect of this sedation technique on mortality in septic shock clearly demonstrated the superiority of sevoflurane over intravenous sedation, with a 20% reduction in both in-hospital and one-year mortality [27].

Another study [28] demonstrated a significant improvement in 7-day overall survival in mice (83.3%) following administration of 1% sevoflurane for 6 hours after induction of abdominal sepsis compared to untreated mice (16.6% in the control group).

Conclusion

The presented clinical observations indicate that prolonged inhalation sedation in the ICU using the AnaConDa device in patients with sepsis has no adverse effect on the cardiovascular system (clinical case 2) and allows to achieve adequate sedation levels without the development of tachyphylaxis. This approach helps to avoid dose escalation in patients requiring multimodal sedation with intravenous anesthetics (clinical cases 1 and 2).

Clearly, prospective randomized clinical trials comparing various sedation strategies are required to gain a full understanding of all aspects of inhalation sedation in the ICU.

References

1. *Потиевская В. И., Заболотских И. Б., Гридчик И. Е., Грицан А. И., Еременко А. А., Козлов И. А., Лебединский К. М., с соавт.* Седация пациентов в отделениях реанимации и интенсивной терапии. *Анестезиология и реаниматология.* 2020; (5): 7–22. *Potievskaya V. I., Zabolotskikh I. B., Gridchik I. E., Gritsan A. I., Eremenko A. A., Kozlov I. A., Lebedinsky K. M., et al.* Sedation of patients in intensive care units. *Russ J Anesthesiol.Reanimatol = Anesteziologiya i Reanimatologiya.* 2020; (5): 7–22. (in Russ.).
DOI: 10.17116/anaesthesiology20200517.
2. *Потиевская В. И., Заболотских И. Б., Гридчик И. Е., Грицан А. И., Еременко А. А., Козлов И. А., Левит А. Л., с соавт.* Седация пациентов в отделениях анестезиологии, реанимации и интенсивной терапии. Методические рекомендации. Анестезиология и реаниматология. 2023; (5): 6–24. *Potievskaya V. I., Zabolotskikh I. B., Gridchik I. E., Gritsan A. I., Eremenko A. A., Kozlov I. A., Levit A. L., et al.* Sedation of patients in intensive care units. Guidelines. *Russ J Anesthesiol.Reanimatol = Anesteziologiya i Reanimatologiya.* 2023; (5): 6–24. (In Russ.).
DOI: 10.17116/anaesthesiology20230516.
3. *Левиков Д. И., Марченков Ю. В., Стрижков Н. А., Засимова М. Я., Шайбакова В. Л., Родионов Е. П.* Седация и неинвазивная масочная вентиляция у пациентов с послеоперационным делирием и острой дыхательной недостаточностью. *Общая реаниматология.* 2020; 16 (2): 4–11. *Levikov D. I., Marchenkov Yu.V., Strizhkov N. A., Zasimova M.Ya., Shaybakova V. L., Rodionov E. P.* Sedation and noninvasive mask ventilation in patients with delirium and acute respiratory failure. *General Reanimatology = Obshchaya reanimatologiya.* 2020; 16 (2): 4–11. (In Russ.&Eng.).
DOI: 10.15360/1813-9779-2020-2-4-11.
4. *Лихванцев В. В., Габитов М. В., Скрипкин Ю. В., Мироненко А. В., Данилов М. П.* Использование компактного устройства с анестетиксберегающим фильтром («ANACONDA») для ингаляционной седации в отделении реанимации и интенсивной терапии. *Вестник интенсивной терапии.* 2014; (3): 38–41. *Likhvantsev V. V., Gabitov M. V., Skripkin Yu.V., Mironenko A. V., Danilov M. P.* Use of a compact device with an anesthetic sparing filter («ANACONDA») for inhalation sedation in the intensive care unit. *Ann Crit Care = Vestnik Intensivnoy Terapii im AI Saltanova.* 2014; (3): 38–41. (In Russ.)
5. *Гребенчиков О. А., Скрипкин Ю. В., Герасименко О. Н., Каданцева К. К., Бачинский А. Л., Берикашвили Л. Б., Лихванцев В. В.* Неанестетические эффекты современных галогенсодержащих анестетиков. *Патология кровообращения и кардиохирургия.* 2020; 24 (2): 26–45. *Grebenchikov O. A., Skripkin Yu.V., Gerasimenko O. N., Kadantseva K. K., Bachinskiy A. L., Berikashvili L. B., Likhvantsev V. V.* Non-anaesthetic effects of modern halogen-containing anaesthetics. *Pathology of Blood Circulation and Cardiac Surgery = Patologiya Krovoobrashcheniya i Kardiokhirurgiya.* 2020; 24 (2): 26–45. (In Russ.).
DOI: 10.21688/1681-3472-2020-2-26-45.
6. *Belletti A., Lerose C. C., Zangrillo A., Landoni G.* Vasoactive-inotropic score: evolution, clinical utility, and pitfalls. *J Cardiothorac Vasc Anesth.* 2021; 35 (10): 3067–3077.
DOI: 10.1053/j.jvca.2020.09.117. PMID: 33069558.
7. *Boldingh Q. J., de Vries F. E., Boermeester M. A.* Abdominal sepsis. *Curr Opin Crit Care.* 2017; 23 (2): 159–166.
DOI: 10.1097/MCC.0000000000000388.
PMID: 28107224.
8. *Buckman S. A., Turnbull I. R., Mazuski J. E.* Empiric antibiotics for sepsis. *Surg Infect. (Larchmt).* 2018; 19 (2): 147–154.
DOI: 10.1089/sur.2017.282. PMID: 29341844.
9. *Ankawi G., Neri M., Zhang J., Breglia A., Ricci Z., Ronco C.* Extracorporeal techniques for the treatment of critically ill patients with sepsis beyond conventional blood purification therapy: the promises and the pitfalls. *Crit Care.* 2018; 22 (1): 262.
DOI: 10.1186/s13054-018-2181-z. PMID: 30360755.
10. *Yuan Z., Zheng R., Chen Q., Wang X.* [Biomarkers for the early diagnosis of sepsis]. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue.* 2019; 31 (3): 381–384. (Chinese).
DOI: 10.3760/cma.j.issn.2095-4352.2019.03.026.
PMID: 30914107.
11. *Candel F. J., Borges Sá M., Belda S., Bou G., Del Pozo J. L., Estrada O., Ferrer R., et al.* Current aspects in sepsis approach. Turning things around. *Rev Esp Quimioter.* 2018; 31 (4): 298–315. PMID: 29938972.
12. *Celis-Rodríguez E., Díaz Cortés J. C., Cárdenas Bolívar Y. R., Carrizosa González J. A., Pinilla D. I., Ferrer Záccaro L. E., Birchenall C., et al.* Evidence-based clinical practice guidelines for the management of sedoanalgesia and delirium in critically ill adult patients. *Med Intensiva. (Engl Ed).* 2020; 44 (3): 171–184.
DOI: 10.1016/j.medin.2019.07.013. PMID: 31492476.
13. *Лещенко Р. Е., Левит А. Л., Давыдова Н. С.* Процедурная седация и/или анальгезия: обзор литературы. *Вестник интенсивной терапии имени А.И. Салтанова.* 2023; (2): 117–129. *Leshchenko R. E., Levit A. L., Davydova N. S.* Procedural sedation and/or analgesia: a literature review. *Ann Crit Care = Vestnik Intensivnoy Terapii im AI Saltanova.* 2023; (2): 117–129. (in Russ.).
DOI: 10.21320/1818-474X-2023-2-117-129.
14. *Чураков В. О., Зайцев А. Ю., Дымова О. В., Дубровин К. В., Жукова С. Г., Матвеева Н. А.* Влияние

интраоперационной седации пропофолом на концентрацию нейромедиаторов (пилотное исследование). *Общая реаниматология*. 2021; 17 (6): 15–19. *Churakov V. O., Zaitsev A. Yu., Dymova O. V., Dubrovin K. V., Zhukova S. G., Matveeva N. A.* Effect of intraoperative propofol-induced sedation on the neurotransmitter levels (pilot study). *General Reanimatology = Obshchaya Reanimatologiya*. 2021; 17 (6): 15–19. (in Russ.&Eng.).
DOI: 10.15360/1813-9779-2021-6-15-19.

15. *Schläpfer M., Piegeler T., Dull R. O., Schwartz D. E., Mao M., Bonini M. G., Z'Graggen B. R., et al.* Propofol increases morbidity and mortality in a rat model of sepsis. *Crit Care*. 2015; 19 (1): 45.
DOI: 10.1186/s13054-015-0751-x. PMID: 25887642.
16. *Roberts R. J., Barletta J. F., Fong J. J., Schumaker G., Kuper P. J., Papadopoulos S., Yogaratnam D., et al.* Incidence of propofol-related infusion syndrome in critically ill adults: a prospective, multicenter study. *Crit Care*. 2009; 13 (5): R169.
DOI: 10.1186/cc8145. PMID: 19874582.
17. *Sanger D. J., Zivkovic B.* Investigation of the development of tolerance to the actions of zolpidem and midazolam. *Neuropharmacology*. 1987; 26 (10): 1513–1518.
DOI: 10.1016/0028-3908(87)90172-9.
PMID: 3683765.
18. *Karabulut G., Bedirli N., Akyürek N., Bağrıaçık E. Ü.* Dose-related effects of dexmedetomidine on sepsis-initiated lung injury in rats. *Braz J Anesthesiol*. 2021; 71 (3): 271–277.
DOI: 10.1016/j.bjane.2021.02.051. PMID: 33845100.
19. *Cioccari L., Luethi N., Bailey M., Shehabi Y., Howe B., Messmer A. S., Proimos H. K., et al; ANZICS Clinical Trials Group and the SPICE III Investigators.* The effect of dexmedetomidine on vasopressor requirements in patients with septic shock: a subgroup analysis of the Sedation Practice in Intensive Care Evaluation [SPICE III] Trial. *Crit Care*. 2020; 24 (1): 441.
DOI: 10.1186/s13054-020-03115-x. PMID: 32678054.
20. *Lankadeva Y. R., Ma S., Iguchi N., Evans R. G., Hood S. G., Farmer D. G. S., Bailey S. R., et al.* Dexmedetomidine reduces norepinephrine requirements and preserves renal oxygenation and function in ovine septic acute kidney injury. *Kidney Int*. 2019; 96 (5): 1150–1161.
DOI: 10.1016/j.kint.2019.06.013. PMID: 31530477.
21. *Likhvantsev V. V., Landoni G., Levikov D. I., Grebenchikov O. A., Skripkin Y. V., Cherpakov R. A.* Sevoflurane versus total intravenous anesthesia for isolated coronary artery bypass surgery with cardiopulmonary bypass: a randomized trial. *J Cardiothorac Vasc Anesth*. 2016; 30 (5): 1221–1227.
DOI: 10.1053/j.jvca.2016.02.030. PMID: 27431595.
22. *Fortis S., Spieth P. M., Lu W. Y., Parotto M., Haitsma J. J., Slutsky A. S., Zhong N., et al.* Effects of anesthetic regimes on inflammatory responses in a rat model of acute lung injury. *Intensive Care Med*. 2012; 38 (9): 1548–1555.
DOI: 10.1007/s00134-012-2610-4. PMID: 22711173.
23. *De Hert S., Vlasselaers D., Barbé R., Ory J. P., Dekegel D., Donnadonni R., Demeere J. L., et al.* A comparison of volatile and non-volatile agents for cardioprotection during on-pump coronary surgery. *Anaesthesia*. 2009; 64 (9): 953–960.
DOI: 10.1111/j.1365-2044.2009.06008.x.
PMID: 19686479.
24. *Kellner P., Müller M., Piegeler T., Eugster P., Booy C., Schläpfer M., Beck-Schimmer B.* Sevoflurane abolishes oxygenation impairment in a long-term rat model of acute lung injury. *Anesth Analg*. 2017; 124 (1): 194–203.
DOI: 10.1213/ANE.0000000000001530.
PMID: 27782948.
25. *Ситкин С. И.* Возможности ингаляционных анестетиков в блокировании чрезмерной воспалительной реакции: обзор литературы. *Вестник интенсивной терапии им. А. И. Салтанова*. 2022; (3): 102–110. *Sitkin S. I.* The potential of inhaled anesthetics in blocking excessive inflammatory response: a literature review. *Ann Crit Care = Vestnik Intensivnoy Terapii im AI Saltanova*. 2022; (3): 102–110. (in Russ.).
DOI: 10.21320/1818-474X-2022-3-102-110.
26. *Kidani Y., Taniguchi T., Kanakura H., Takemoto Y., Tsuda K., Yamamoto K.* Sevoflurane pretreatment inhibits endotoxin-induced shock in rats. *Anesth Analg*. 2005; 101 (4): 1152–1156.
DOI: 10.1213/01.ane.0000167768.55939.e1.
PMID: 16192536.
27. *Bellgardt M., Bomberg H., Herzog-Niescery J., Dasch B., Vogelsang H., Weber T. P., Steinfort C., et al.* Survival after long-term isoflurane sedation as opposed to intravenous sedation in critically ill surgical patients: Retrospective analysis. *Eur J Anaesthesiol*. 2016; 33 (1): 6–13.
DOI: 10.1097/EJA.0000000000000252.
PMID: 25793760.
28. *Liu D., Mei L., Zhao P.* Immunomodulatory effects of anaesthetic sevoflurane in septic mouse model. *Saudi J Biol Sci*. 2021; 28 (5): 2733–2738.
DOI: 10.1016/j.sjbs.2021.03.023. PMID: 34012317.

Received 13.11.2023
Accepted 21.11.2024

https://doi.org/10.15360/1813-9779-2025-1-55-61

Heterogeneity of NeuN Protein Distribution as a Marker of Morphological Personalization of Cerebral Cortex Neurons: an Experimental Study

Arkady M. Golubev*, Maxim A. Lyubomudrov*, Anastasia S. Babkina, Zoya I. Tsokolaeva

V. A. Negovsky Research Institute of General Reanimatology,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia

For citation: *Arkady M. Golubev, Maxim A. Lyubomudrov, Anastasia S. Babkina, Zoya I. Tsokolaeva.* Heterogeneity of NeuN Protein Distribution as a Marker of Morphological Personalization of Cerebral Cortex Neurons: an Experimental Study. *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 55–61. https://doi.org/10.15360/1813-9779-2025-1-55-61 [In Russ. and Engl.]

***Correspondence to:** Arkady M. Golubev, arkadygolubev@mail.ru; Maxim A. Lyubomudrov, lyubomudrov@fnkcrr.ru

Summary

Aim. To identify personalized morphological neuronal phenotypes based on the distribution pattern of the neuronal protein NeuN in the cerebral cortex layers.

Materials and Methods. A histologic study of the cerebral cortex was performed in rats (*N*=10). Tissue sections were stained with hematoxylin and eosin, and the neuronal nuclear protein NeuN was visualized by immunohistochemical staining. Analysis was performed by microscopy and image analysis software.

Results. NeuN immunohistochemical staining revealed distinct localization and intensity patterns within cortical neurons. Contrary to the definition of NeuN as a nuclear neuronal protein, its localization was observed in both the nucleus and cytoplasm in most neurons. The following neuronal phenotypes were identified based on NeuN staining patterns:

1) Neurons with stained nuclei but unstained cytoplasm;
2) Neurons with stained cytoplasm but unstained nuclei;
3) Neurons with stained nuclei and cytoplasm;
4) Fully stained neurons with no visible nuclei;
5) Neurons with stained processes (dendrites/axons).

A significant difference was found between mean intensity of NeuN-positive neurons depending on the localization in the layers of the cerebral cortex.

Conclusion. Given the critical biological role of NeuN, the identified neuronal phenotypes based on NeuN localization warrant further research as they may reflect the functional states of neurons. The interpretation of the absence of NeuN staining as a marker of neuronal damage is not scientifically justified. Future studies using NeuN immunohistochemical staining should consider not only the total number of NeuN-positive neurons, but also their distinct phenotypes.

Keywords: NeuN protein; personalized neuronal phenotyping; neuronal phenotypes; cerebral cortex; immunohistochemistry; morphology; morphometry.

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

Information about the authors:
Arkady M. Golubev: ORCID 0000-0002-3165-0378
Maksim A. Lyubomudrov: ORCID 0000-0002-1735-592X
Anastasia S. Babkina: ORCID 0000-0003-1780-9829
Zoya I. Tsokolaeva: ORCID 0000-0003-2441-6062

Introduction

The NeuN protein was discovered during research on monoclonal antibodies (mAb) targeting brain cell nuclei. One of these antibodies, mAb A60, recognizes a nuclear protein specific to vertebrate neurons. This protein has been named NeuN (neuronal nuclei). Immunohistochemically detectable NeuN first appears when a neuron exits the cell cycle or begins terminal differentiation [1].

For a long time, the protein to which the A60 antibody binds was unknown. NeuN was first identified as Fox-3, also known as Rbfox3 [2], in 2009. The mammalian genome contains three genes: Fox-1, Fox-2, and Fox-3. The Fox mammalian proteins play a role in regulating mRNA splicing. An alternative name for these three proteins is hexaribonucleotide-binding protein 1, 2, and 3 (HRNBP1, HRNBP2, and HRNBP3).

Thus, NeuN (Fox-3, Rbfox3, or hexaribonucleotide-binding protein-3) is a neuronal nuclear antigen that is widely used as a biomarker for neurons.

This neuron-specific nuclear protein is consistently expressed in most postmitotic neurons of the vertebrate nervous system. The absence of NeuN staining has been interpreted as a marker of neuronal damage in several studies [3–6]. Studies using primary antibodies against NeuN have been performed on both experimental and human autopsy

material. Literature data highlight the potential of NeuN in the study of neuronal responses to injury, including in interspecies comparative studies [7].

However, many studies question the role of NeuN as a marker of intact neurons, noting that its staining is variable and may be absent under certain pathological or physiological conditions [8]. It has been shown that the presence or absence of immunohistochemical (IHC) reactions with primary antibodies against NeuN depends on the phosphorylation status of NeuN, which serves as an epitope for Rbfox3, a member of the Rbfox1 splicing factor family [9]. Thus, the absence of NeuN staining may not indicate neuronal death or complete loss of neuronal expression, but rather a reduction in NeuN protein expression or a loss of NeuN antigenicity [10].

Previously, we observed significant variability in NeuN staining in the cortical neurons of patients who died of COVID-19 [11]. However, the factors leading to decreased protein expression or loss of antigenicity remain poorly understood. Furthermore, there is limited information on the relationship between neuronal functional activity and the characteristics of NeuN staining and localization.

The aim of the study was to identify personalized neuronal morphological phenotypes based on the distribution patterns of the neuronal protein NeuN in the cerebral cortex layers.

Materials and Methods

Ten brain samples were collected from male Wistar rats weighing 200–250 g (N=10) for histologic analysis. Experiments were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes. Rats were euthanized by cervical dislocation under general anesthesia with intraperitoneal administration of zolazepam (20 mg/kg) and xylazine (5 mg/kg). Brain tissue was fixed in 10% neutral formalin and processed by standard paraffin embedding techniques. Tissue sections of 4 μm thickness were stained with hematoxylin and eosin.

The nuclear neuronal protein NeuN was visualized by immunohistochemical staining. Tissue sections were deparaffinized in xylene and rehydrated in graded ethanol. Heat-induced epitope retrieval was performed in citrate buffer, pH 6.0 (Target Retrieval Solution, DAKO, Glostrup, Denmark). Sections were cooled, washed three times in distilled water, and rinsed three times for 5 minutes each in phosphate-buffered saline (PBS) with Tween (IHC Wash Buffer + Tween, Cell Marque, Rocklin, CA, USA). Endogenous peroxidase activity was blocked by incubating sections in 3% hydrogen peroxide for 10 minutes. To prevent non-specific binding of primary or secondary antibodies to tissue proteins, a protein blocking solution (Protein Block Serum-free, Abcam, Cambridge, UK) was applied for 30 minutes.

Sections were incubated for 1 hour at 37°C with primary antibodies against NeuN (Abcam, Cambridge, UK) diluted in antibody diluent (Abcam, Cambridge, UK). After incubation, sections were washed three times for 5 minutes each in PBS. Antibody-antigen binding was detected using a commercial kit (Diagnostic BioSystems, Netherlands) containing secondary antibodies and chromogenic substrate (DAB). After washing in PBS, the sections were counterstained with hematoxylin, rinsed in running tap water, dehydrated in graded ethanol, and mounted under cover slips.

Samples were analyzed using a Nikon Eclipse Ni-U microscope. The mean staining intensity of NeuN-positive neurons was determined based on the Mean Density parameter using NIS-Elements BR image analysis software.

Statistical analysis was performed with IBM SPSS Statistics 29.0. A nonparametric statistical method using the Kruskal-Wallis test was used for comparative analysis between groups. Post hoc analysis was performed using Dunn's test with Bonferroni correction. Differences were considered statistically significant at $P<0.05$ (two-tailed test). Statistical data were reported as medians with interquartile range.

Results

In the small neurons of the first cortical layer, NeuN was localized to the periphery of the nuclei.

In the nuclei of the second layer, the nuclear periphery was stained, while the central part of the nuclei showed diffuse and less intense staining. Weak and diffuse staining was observed in the cytoplasm of the neurons. In some neurons, the nuclei demonstrated diffuse and intense staining. A positive reaction for NeuN was observed in the nucleoli of the nuclei. In some neurons, both the nucleus and the cytoplasm were very weakly stained or not stained at all.

The nuclei of most pyramidal neurons in the third layer showed intense diffuse staining. Neurons with nuclei of low staining intensity were also observed. Cytoplasmic staining was low intensity and diffuse. Positive staining was detected in the initial segments of neuronal processes, including dendrites and axons.

Small nuclei were observed in the fourth cortical layer with more intense staining at the periphery. The hyaloplasm was uniformly stained with moderate intensity. In some neurons, the nuclei and cytoplasm were either unstained or showed minimal staining intensity.

In the fifth layer, the nuclei of many large pyramidal neurons showed variable staining intensity. In the cytoplasm, reaction products were represented by a small number of fine granules. Diffuse staining, occasionally combined with stained granules, pre-

dominated. In some large neurons, both nuclei and cytoplasm were either unstained or showed low intensity staining. In some neurons, the initial segments of dendrites and axons showed low intensity staining. The nuclei and cytoplasm of smaller neurons displayed variable staining intensities.

The sixth layer of polymorphic cells was characterized by variable staining of neuronal nuclei and cytoplasm. Numerous neuronal nuclei showed peripheral localization of NeuN, resulting in ring-like staining patterns along the nuclear periphery after immunohistochemical reaction. Many weakly stained or unstained neuronal nuclei were observed at the border with the white matter of the brain. Moderate or less intense staining was observed in the initial segments of neuronal axons and dendrites.

Based on the staining characteristics of NeuN in cortical neurons, the following phenotypes were identified (Fig. 1):

1) Neurons with stained nuclei but unstained cytoplasm;

2) Neurons with stained cytoplasm but unstained nuclei;

3) Neurons with stained nuclei and cytoplasm;

4) Fully stained neurons with no visible nuclei;

5) Neurons with stained processes (dendrites/axons).

The staining intensity of NeuN-positive neurons varied depending on the cortical layer (Fig. 2, Table). A comparative analysis of the cortical layers based on the mean NeuN staining intensity revealed differences shown in the Table.

Discussion

The cortical neuron phenotypes described above, based on the localization of IHC reactions with NeuN antibodies, have been sporadically men-

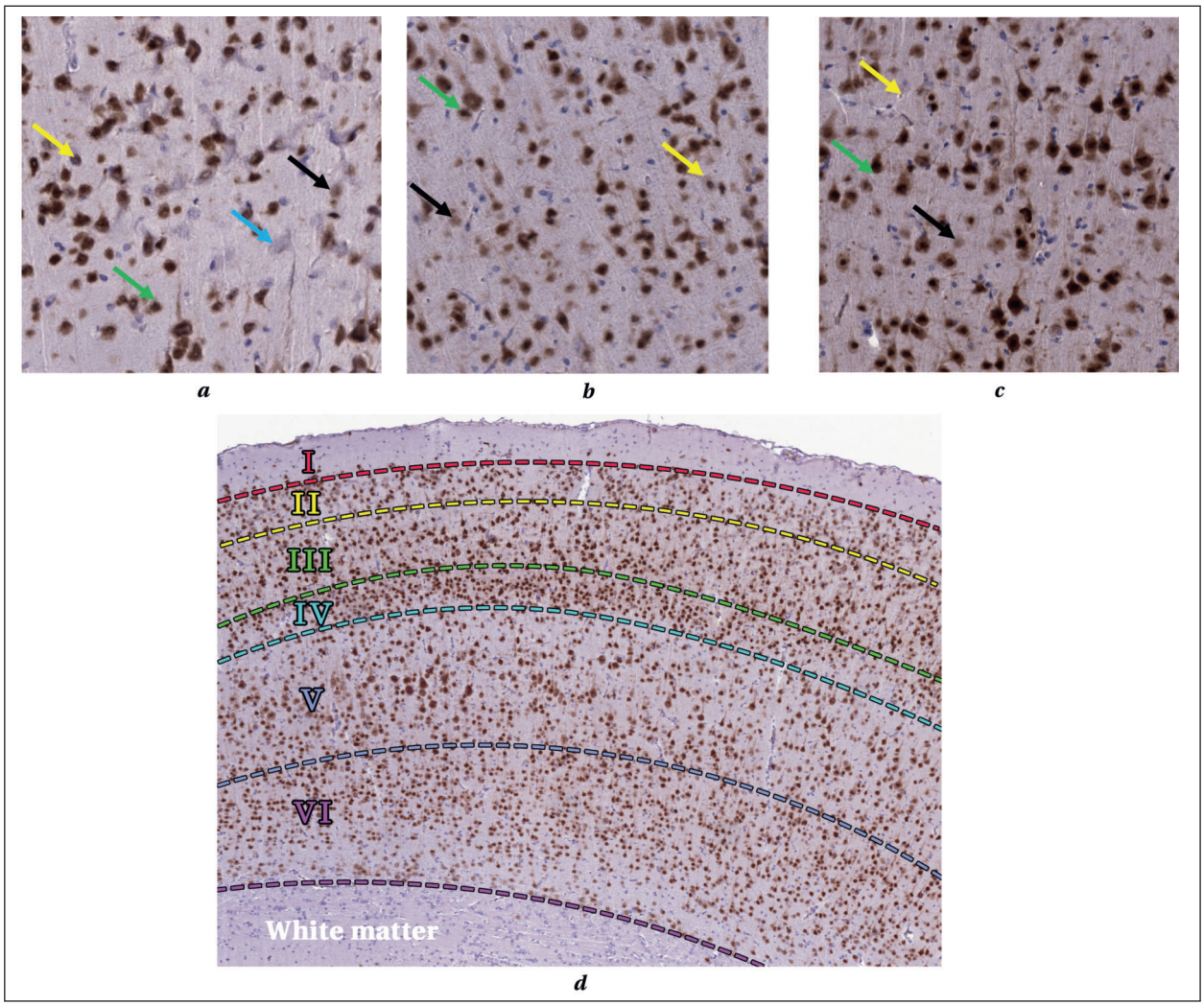

Fig. 1. Localization of NeuN in rat cerebral cortex neurons.
Note. Immunohistochemical (IHC) staining, magnification ×20. Panels *(a–c)* show different staining intensities ranging from weak to strong. *Green arrows*: Neurons with positive IHC staining in both nuclei and cytoplasm. *Yellow arrows*: Neurons with positive IHC staining in nuclei only. *Black arrows*: Neurons with positive IHC staining but without visible nuclear borders. *Blue arrows*: Unstained neurons. Panel *d*: colored lines indicate the boundaries between cortical layers, with Roman numerals indicating the corresponding layers.

tioned in previous studies. In particular, some attempts have been made to explain the variability in NeuN staining and some results have been obtained.

The most common phenotype identified in our study includes neurons with both nuclear and cytoplasmic staining. The neuronal nuclear protein NeuN is known to associate with DNA, and according to Y. S. Lin et al. IHC reactions for NeuN are predominantly observed in nuclear regions and to a lesser extent in the perinuclear cytoplasm [12]. This nuclear and cytoplasmic staining may be due to the subcellular localization of different NeuN/Rbfox3 subtypes. Specifically, the 46-kDa subtype is primarily localized in the nucleus, whereas the 48-kDa subtype is predominantly distributed in the cytoplasm [13]. In certain neurons, such as cerebellar granule cells, nuclear staining may be absent in afferent autonomic neurons while the cytoplasm shows a positive IHC reaction [14].

The varying intensity of NeuN staining and the presence of NeuN-negative neurons cannot be interpreted as an indication of neuronal damage alone, but may reflect differences in neuronal functional activity. It has been suggested that the intensity of NeuN staining in the nucleus is related to the chromatin state. Studies have shown that brain cell nuclei in mice expressing high levels of NeuN/FOX3 have decondensed chromatin compared to nuclei with weak or absent staining [15].

The presence of NeuN-negative neurons in the brain, independent of pathological factors, is supported by the findings of F. A. Azevedo et al. who demonstrated that the adult human brain contains 86.1±8.1 billion NeuN-positive cells («neurons») and 84.6±9.8 billion NeuN-negative cells [16].

Further evidence for a stable subpopulation of NeuN-negative neurons in the lateral neocortex was provided by M. L. Hernandez et al. who reported that these neurons may be more susceptible to late cell membrane damage [17].

In a study of substantia nigra neurons in intact laboratory animals (rats) not exposed to pathologic factors, significant variability in NeuN staining was found. This study identified not only NeuN-positive neurons, but also unstained neurons and neurons with weak NeuN staining, as well as different subcellular compartmentalization of NeuN. The authors concluded that morphometric assessment of NeuN expression cannot be reliably used as a neuronal marker in the substantia nigra [18].

A study by I. Unal-Cevik et al. examined NeuN in mouse brains 6 hours after cerebral ischemia, comparing immunohistochemical (IHC) and Western blot analyses. Their results showed that brain samples with no IHC reaction did not show reduced protein levels, suggesting that the decrease in NeuN IHC staining intensity was due to metabolic disturbances rather than neuronal damage or a reduction in neuronal number [19].

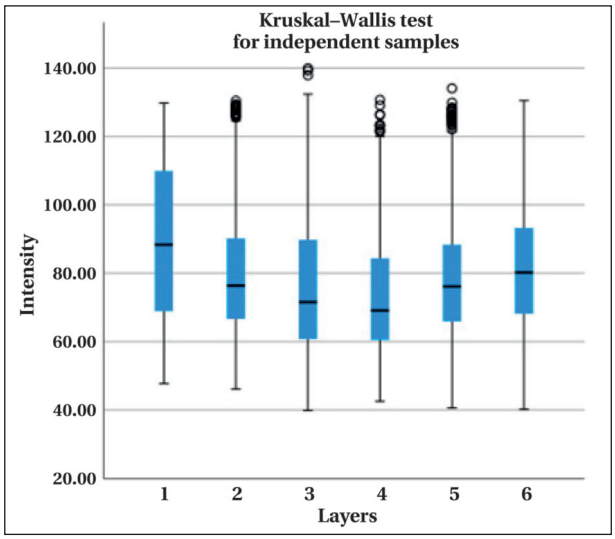

Fig. 2. Staining intensity values of NeuN-positive neurons in the layers of the rat cerebral cortex.

Table. Average staining intensity of NeuN-positive cells in different layers of rat cerebral cortex (median, *Me [Q1–Q3]*).

Layers compared	Average staining intensity		*P*-value
4–3	69 (60–84)	71 (60–89)	0.033*
4–5	69 (60–84)	76 (65–88)	<0.001*
4–2	69 (60–84)	76 (66–90)	<0.000*
4–6	69 (60–84)	68 (80–93)	<0.000*
4–1	69 (60–84)	88 (68–110)	<0.000*
3–5	71 (60–89)	76 (65–88)	<0.000*
3–2	71 (60–89)	76 (66–90)	<0.000*
3–6	71 (60–89)	68 (80–93)	<0.000*
3–1	71 (60–89)	88 (68–110)	<0.000*
5–2	76 (65–88)	76 (66–90)	1.000
5–6	76 (65–88)	68 (80–93)	<0.000*
5–1	76 (65–88)	88 (68–110)	<0.000*
2–6	76 (66–90)	68 (80–93)	0.015*
2–1	76 (66–90)	88 (68–110)	<0.001*
6–1	68 (80–93)	88 (68–110)	0.123

Note. * — significant differences.

Research has suggested that the number of NeuN-positive neurons may be influenced by age and sex [20–22]. For example, A. Sugiura et al. observed a decrease in NeuN-containing hypothalamic neurons with advancing age [22]. However, our examination of the cerebral cortex in COVID-19 non-survivors did not show any effect of sex or age on NeuN staining [11].

L. Luijerink et al. observed weaker IHC reactions in paraffin-embedded human brain sections compared to those from experimental animals. They attributed this difference to the methods of fixation and histological preparation [23]. However, other studies using experimental material have shown significant variability in neuronal staining regardless of the fixation technique employed [18].

Studies have examined NeuN expression in various pathological conditions and injuries. L. T. McPhail et al. found that transection of peripheral nerves in rats and mice resulted in almost complete loss of NeuN in facial motor neurons

within three days of injury. This depletion persisted for up to 28 days, although the neurons remained viable [24]. In cases of sudden perinatal death, NeuN staining was significantly reduced or absent. Notably, neurons with reduced NeuN labeling showed no evidence of apoptosis. Furthermore, a strong association between NeuN depletion in the fetal brain neurons and maternal smoking was observed [25].

In another study, Anderson et al. observed an increase in NeuN expression in the nuclei and cytoplasm of neurons in the dorsal root ganglia on an experimental model of adjuvant-induced arthritis [26]. This finding led the investigators to suggest that NeuN may be involved in the activation of nociceptive neurons and contribute to pain signaling.

Conclusion

Given the important biological role of the NeuN protein, researchers should closely examine the phenotypes of neurons characterized by different NeuN localizations, as these patterns may reflect the functional state of the neurons. The absence of NeuN staining should not be interpreted as evidence of neuronal damage, as there is no scientific justification for this assumption. The studies using immunohistochemical staining and NeuN antibodies should consider and analyze not only the total number of NeuN-positive neurons, but also their distinct phenotypic variations. Based on the results showing significant differences in NeuN staining intensity across cortical layers, future studies should explore the identified neuronal phenotypes as a potential criterion for personalized profiling of cortical layers in the brain.

References

1. *Mullen R. J., Buck C. R., Smith A. M.* NeuN, a neuronal specific nuclear protein in vertebrates. *Development.* 1992; 116 (1): 201–211. DOI: 10.1242/dev.116.1.201. PMID: 1483388.
2. *Kim K. K., Adelstein R.S, Kawamoto S.* Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. *J Biol Chem.* 2009; 284 (45): 31052–31061. DOI: 10.1074/jbc.M109.052969 PMID: 19713214.
3. *Alekseeva O. S., Guselnikova V. V., Beznin G. V., Korzhevsky D. E.* Prospects for the application of neun nuclear protein as a marker of the functional state of nerve cells in vertebrates. *J Evol Biochem. Phys.* 2015; 51: 357–369. DOI: 10.1134/S0022093015050014.
4. *Shen C. C., Yang Y. C., Chiao M. T., Cheng W. Y., Tsuei Y. S., Ko J. L.* Characterization of endogenous neural progenitor cells after experimental ischemic stroke. *Curr Neurovasc Res.* 2010; 7 (1): 6–14. DOI: 10.2174/156720210790820208.
5. *Davoli M. A., Fourtounis J., Tam J., Xanthoudakis S., Nicholson D., Robertson G. S., Ng G. Y., Xu D.* Immunohistochemical and biochemical assessment of caspase-3 activation and DNA fragmentation following transient focal ischemia in the rat. *Neuroscience.* 2002; 115 (1): 125–136. DOI: 10.1016/S0306-4522(02)00376-7. PMID: 12401327.
6. *Sugawara T., Lewén A., Noshita N., Gasche Y., Chan P. H.* Effects of global ischemia duration on neuronal, astroglial, oligodendroglial, and microglial reactions in the vulnerable hippocampal CA1 subregion in rats. *J Neurotrauma.* 2002; 19: 85–98. DOI: 10.1089/089771502753460268. PMID: 11852981.
7. *Alekseeva O. S., Gusel'nikova V. V., Beznin G. V., Korzhevskii D. E.* Prospects of the nuclear protein NeuN application as an index of functional state of the vertebral nerve cells]. *Zh Evol Biokhim Fiziol.* 2015; 51 (5): 313–323. (in Russ.). PMID: 26856070.
8. *Duan W., Zhang Y. P., Hou Z., Huang C., Zhu H., Zhang C. Q., Yin Q.* Novel insights into NeuN: from neuronal marker to splicing regulator. *Mol Neurobiol.* 2016; 53: 1637–1647. DOI: 10.1007/s12035-015-9122-5. PMID: 25680637.
9. *Kim K. K., Adelstein R. S., Kawamoto S.* Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. *J Biol Chem.* 2009; 284: 31052–31061. DOI: 10.1074/jbc.M109.052969. PMID: 19713214.
10. *Unal-Cevik I., Kilinç M., Gürsoy-Ozdemir Y., Gurer G., Dalkara T.* Loss of NeuN immunoreactivity after cerebral ischemia does not indicate neuronal cell loss: A cautionary note. *Brain Res.* 2004; 1015: 169–174. DOI: 10.1016/j.brainres.2004.04.032. PMID: 15223381.
11. *Babkina A. S., Yadgarov M. Y., Lyubomudrov M. A., Ostrova I. V., Volkov A. V., Kuzovlev A. N., Grechko A. V., et al.* Morphologic findings in the cerebral cortex in COVID-19: association of microglial changes with clinical and demographic variables. *Biomedicines.* 2023; 11 (5): 1407. DOI: 10.3390/biomedicines11051407. PMID: 37239078.
12. *Lin Y. S., Kuo K. T., Chen S. K., Huang H. S.* RBFOX3/NeuN is dispensable for visual function. *PLoS One.* 2018; 13 (2): e0192355. DOI: 10.1371/journal.pone.0192355. PMID: 29401485.
13. *Maxeiner S., Glassmann A., Kao H. T., Schilling K.* The molecular basis of the specificity and cross-reactivity of the NeuN epitope of the neuron-specific splicing regulator, Rbfox3. *Histochem Cell Biol.* 2014; 141: 43–55. DOI: 10.1007/s00418-013-1159-9. PMID: 24150744.
14. *Van Nassauw L., Wu M., De Jonge F., Adriaensen D., Timmermans J. P.* Cytoplasmic, but not nuclear, expression of the neuronal nuclei (NeuN) antibody is an exclusive feature of Dogiel type II neurons in the guinea-pig gastrointestinal tract. *Histochem. Cell Biol.* 2005; 124: 369–377. DOI: 10.1007/s00418-005-0019-7. PMID: 16049694.
15. *Yu P., McKinney E. C., Kandasamy M. M., Albert A. L., Meagher R. B.* Characterization of brain cell nuclei with decondensed chromatin. *Dev Neurobiol.* 2015; 75 (7): 738–756. DOI: 10.1002/dneu.22245. PMID: 25369517.
16. *Azevedo F. A., Carvalho L. R., Grinberg L. T., Farfel J. M., Ferretti R. E., Leite R. E., Jacob Filho W., et al.* Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol.* 2009; 513 (5): 532–541. DOI: 10.1002/cne.21974. PMID: 19226510.
17. *Hernandez M. L., Chatlos T., Gorse K. M., Lafrenaye A. D.* Neuronal membrane disruption occurs late following diffuse brain trauma in rats and involves a subpopulation of NeuN negative cortical neurons. *Front Neurol.* 2019; 10: 1238. DOI: 10.3389/fneur.2019.01238. PMID: 31824411.
18. *Cannon J. R., Greenamyre J. T.* NeuN is not a reliable marker of dopamine neurons in rat substantia nigra. *Neurosci Lett.* 2009; 464 (1): 14–17. DOI: 10.1016/j.neulet.2009.08.023. PMID: 19682546.
19. *Unal-Cevik I., Kilinç M., Gürsoy-Ozdemir Y., Gurer G., Dalkara T.* Loss of NeuN immunoreactivity after cerebral ischemia does not indicate neuronal cell loss: a cautionary note. *Brain Res.*

2004; 1015 (1–2): 169–174.
DOI: 10.1016/j.brainres.2004.04.032.
PMID: 15223381.

20. *Yagi S., Splinter J. E.J., Tai D., Wong S., Wen Y., Galea L. A. M.* Sex differences in maturation and attrition of adult neurogenesis in the hippocampus. *eNeuro*. 2020; 7 (4): ENEURO.0468-19.2020.
DOI: 10.1523/ENEURO.0468-19.2020.
PMID: 32586842.
21. *Demarest T. G., Waite E. L., Kristian T., Puche A. C., Waddell J., McKenna M. C., Fiskum G.* Sex-dependent mitophagy and neuronal death following rat neonatal hypoxia-ischemia. *Neuroscience*. 2016; 335: 103–113.
DOI: 10.1016/j.neuroscience.2016.08.026.
PMID: 27555552.
22. *Sugiura A., Shimizu T., Kameyama T., Maruo T., Kedashiro S., Miyata M., Mizutani K., et al.* Identification of Sox2 and NeuN double-positive cells in the mouse hypothalamic arcuate nucleus and their reduction in number with aging. *Front Aging Neurosci*. 2021; 12: 609911.
DOI: 10.3389/fnagi.2020.609911.
PMID: 33776740.
23. *Luijerink L., Waters K. A., Machaalani R.* Immunostaining for NeuN does not show all mature and healthy neurons in the human and pig brain: focus on the hippocampus. *Appl Immunohistochem Mol Morphol*. 2021; 29: e46–e56.
DOI: 10.1097/PAI.0000000000000925.
PMID: 33710124.
24. *McPhail L. T., McBride C. B., McGraw J., Steeves J. D., Tetzlaff W.* Axotomy abolishes NeuN expression in facial but not rubrospinal neurons. *Exp Neurol*. 2004; 185 (1): 182–190.
DOI: 10.1016/j.expneurol.2003.10.001.
PMID: 14697329.
25. *Lavezzi A. M., Corna M. F., Matturri L.* Neuronal nuclear antigen (NeuN): a useful marker of neuronal immaturity in sudden unexplained perinatal death. *J Neurol Sci*. 2013; 329 (1–2): 45–50.
DOI: 10.1016/j.jns.2013.03.012. PMID: 23570982.
26. *Anderson M. B., Das S., Miller K. E.* Subcellular localization of neuronal nuclei (NeuN) antigen in size and calcitonin gene-related peptide (CGRP) populations of dorsal root ganglion (DRG) neurons during acute peripheral inflammation. *Neurosci Lett*. 2021: 24; 760: 135974.
DOI: 10.1016/j.neulet.2021.135974.
PMID: 34146639.

Received 18.09.2024
Accepted 17.12.2024

https://doi.org/10.15360/1813-9779-2025-1-62-74

Emergency Ultra-Deep Hypothermia in Cardiac Arrest Induced by Blood Loss (Experimental Study on Nonhuman Primates)

Victor A. Reva[1]*, Anastasia R. Samakaeva[1], Daniil A. Shelukhin[2], Sergey V. Orlov[3], Vladimir D. Potemkin[1], Dmitry V. Bulgin[3], Galina Y. Gracheva[4], Alexey V. Shchegolev[1]

[1] S.M. Kirov Military Medical Academy,
6 Academician Lebedev Str., B, 194044 St. Petersburg, Russia
[2] My Medical Center,
4 A Finlandsky Prospekt, 191186 Str. Petersburg, Russia
[3] Kurchatov Complex for Medical Primatology, National Research Center, Kurchatov Institute,
177 Academician Lapina Str., Vesyoloye vil., 354376 Sochi, Russia
[4] Duocor Multidisciplinary Scientific and Educational Center,
10 Lvovskaya Str., 195176 St. Petersburg, Russia

For citation: *Victor A. Reva, Anastasia R. Samakaeva, Daniil A. Shelukhin, Sergey V. Orlov, Vladimir D. Potemkin, Dmitry V. Bulgin, Galina Y. Gracheva, Alexey V. Shchegolev.* Emergency Ultra-Deep Hypothermia in Cardiac Arrest Induced by Blood Loss (Experimental Study on Nonhuman Primates). *Obshchaya Reanimatologiya = General Reanimatology.* 2025; 21 (1): 62–74. https://doi.org/10.15360/1813-9779-2025-1-62-74 [In Russ. and Engl.]

***Correspondence to:** Victor A. Reva, vreva@mail.ru

Summary

The survival rate of critically injured individuals with severe blood loss and cardiac arrest is close to zero.

Aim. To evaluate the feasibility of using emergency ultra-deep hypothermia (EUDH) in an experimental model of cardiac arrest induced by blood loss in nonhuman primates.

Materials and Methods. Five male olive baboons (*Papio anubis*), weighing 19.8 (18.8–23.8) kg, were subjected to severe blood loss leading to cardiac arrest. After 1 minute of observation and 3 minutes of cardiopulmonary resuscitation (CPR), aortic arch cooling was initiated using extracorporeal membrane oxygenation (ECMO) with a 4°C solution to achieve a nasopharyngeal temperature of 10°C. Whole-body cooling followed until a rectal temperature of 16°C was reached. Balloon catheters were used to disconnect the upper and lower halves of the body. Once the target temperatures were reached, the ECMO circuit was turned off and an open laparotomy was performed to simulate damage control strategies. One hour after cardiac arrest, slow rewarming began at a rate of 1°C per 10 minutes to 1°C per hour, accompanied by reinfusion of previously collected blood. After return of spontaneous circulation (ROSC), sustained breathing, and tracheal extubation, the animals were transferred to a vivarium.

Results. During deep hypothermia, cerebral oximetry values remained within normal limits in all animals. Sustained ROSC was recorded in 4 of 5 animals at temperatures between 22–25°C. Two animals survived to the end of the experiment but died after extubation, 44 and 19 hours after the start of the experiment. Cooling rates for survivors were 7–11 minutes compared to 23–37 minutes for non-survivors. Causes of death included systemic hypoperfusion with subsequent reperfusion syndrome as evidenced by progressive lactate elevation, elevated creatine phosphokinase levels, cerebral edema, myocardial ischemia, and transient coagulopathy.

Conclusion. EUDH supports adequate cerebral perfusion during temporary circulatory arrest. Recovery of cardiac activity and, in some cases, awakening are achievable during the rewarming phase. Causes of death and possible corrective measures require further investigation.

Keywords: ultra-deep hypothermia; cardiac arrest; blood loss; reperfusion; primate experiment; ECMO

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

Funding. This study was conducted with the financial support of the Russian Science Foundation under Project No. 23-25-00310.

Information about the authors:
Victor A. Reva: ORCID 0000-0001-6705-9849
Anastasia R. Samakaeva: ORCID 0009-0001-4397-0870
Daniil A. Shelukhin: ORCID 0000-0002-2510-002X
Sergey V. Orlov: ORCID 0000-0001-6080-8042
Vladimir D. Potemkin: ORCID 0009-0009-9969-3524
Dmitry V. Bulgin: ORCID 0000-0003-1739-8505
Galina Y. Gracheva: ORCID 0009-0007-0589-1753
Alexey V. Shchegolev: ORCID 0000-0001-6431-439X

Introduction

Severe trauma with ongoing bleeding and the development of cardiac arrest is overwhelmingly fatal. Current resuscitation methods are largely ineffective in traumatic cardiac arrest (TCA). The success rate of standard cardiopulmonary resuscitation (CPR), including closed chest compressions, is extremely low [1, 2]. Even when thoracotomy is performed to place an aortic cross-clamp and perform open chest cardiac massage, survival rates do not exceed 10% [3].

Regardless of the cause of cardiac arrest, irreversible changes begin to occur in the brain within 5 minutes and in the heart within 20 minutes [4, 5]. Since the 1950s and 1960s, researchers have sought ways to reduce the oxygen demand of vital organs [6, 7]. Building on the pioneering work of V. A. Negovsky, who studied mechanical circulatory support and the use of hypothermia [5, 8, 9], P. Safar and S. Tisherman introduced the concept of emergency preservation and resuscitation (EPR) [10–12].

The essence of the method is emergency profound (ultra-deep) hypothermia (EPH), which is achieved by rapid cooling of the upper body (head and heart) followed by whole body cooling to a temperature of 10–16°C. This is achieved by infusing cooled (4°C) saline into the vascular system. According to its proponents, this approach minimizes oxygen demand to vital organs, allowing surgeons to perform life-saving surgery followed by resuscitation and gradual rewarming.

EPR has been successfully implemented in animal studies with high survival rates [13, 14], paving the way for regulatory approval of a pilot clinical trial in trauma patients [15].

The described method has several limitations. It requires a wide thoracotomy approach to clamp the major vessels (aorta and inferior vena cava [IVC]) and to place the return cannula (directed toward the heart in the descending aorta) and the drainage cannula (in the right atrial appendage). This creates an isolated «upper» circuit for rapid cooling of the brain. However, one of the critical tasks of EPH — rapid cannulation and isolation of the upper circuit — is difficult to achieve with this protocol. Thoracotomy and open cannulation of major vessels can take more than 5 minutes, which is critical to maintaining cerebral perfusion. In addition, the extensive thoracotomy itself introduces another source of blood loss due to the subsequent development of coagulopathy.

It was hypothesized that the principles of EPH could be implemented in a minimally invasive manner. This could be achieved by combining resuscitative endovascular balloon occlusion of the aorta (REBOA) with open cannulation of the right brachial artery, allowing selective perfusion of the aortic arch [16].

The aim of this study was to experimentally evaluate the feasibility of using a modified EPH technique during hemorrhagic shock-induced cardiac arrest in non-human primates.

Materials and Methods

The study was conducted at the Kurchatov Medical Primatology Complex of the National Research Center «Kurchatov Institute» (MPC NRC «Kurchatov Institute»), Sochi, with the approval of the local ethics committee (Protocol No. 279, dated June 27, 2023).

The study included five male olive baboons (*Papio anubis*) with a mean age of 5.6 years (range: 5.3–10.8 years) and a mean body weight of 19.8 kg (range: 18.8–23.8 kg). The selection of these animals (non-human primates) was driven by the need to evaluate the development of functional, including cognitive, impairments resulting from macro- and/or microstructural changes in the brain under conditions of deep hypothermia. All procedures involving baboons were performed in accordance with generally accepted legal and ethical standards for animal care, according to standard operating procedures adopted at the MPC NRC «Kurchatov Institute». Compliance with the Decision of the Council of the EEC dated November 3, 2016, No. 81 «On Approval of the Rules of Good Laboratory Practice for the Eurasian Economic Union in the Field of Medicinal Products Handling», as well as GOST 33044–2014 «Principles of Good Laboratory Practice» and GOST 33218–2014 Interstate Standard «Guidelines for the Housing and Care of Laboratory Animals. Rules for housing and care of non-human primates» was ensured.

The experimental protocol consisted of several key phases: animal preparation, preparation and priming of the ECMO circuit, simulation of blood loss and cardiac arrest, emergency hypothermia, and subsequent rewarming of the animal (Fig. 1).

Animal Preparation and Vascular Catheterization. After 24 hours of fasting, animals were sedated with intramuscular tiletamine and zolazepam (Telazol, Zoetis®, USA) at a dose of 15–20 mg/kg. They were transported to the operating room where tracheal intubation was performed followed by mechanical ventilation. Animals were positioned supine on the operating table with limbs extended. A 5 Fr introducer was placed in the left jugular vein to facilitate maintenance of intravenous fluid therapy and blood sampling. Both femoral arteries were catheterized with 7 Fr introducers: one for invasive BP monitoring and placement of a balloon catheter for thoracic aortic occlusion (MIT catheter, Zheleznodorozhny, Russia) and the other for insertion of a 12 Fr arterial return cannula.

In addition, the right femoral vein was catheterized to insert a second balloon catheter to occlude the inferior vena cava (IVC) at its junction with the right atrium, ensuring complete isolation of the upper circulatory compartment.

A 5 cm incision was made in the right axilla to expose the right brachial artery and vein. Through these, a second 15 Fr arterial return cannula was inserted with its tip positioned in the aortic arch, and a 17 Fr drainage cannula was inserted with its tip positioned in the superior vena cava (SVC) lumen (all cannulae from Biomedicus, Medtronic, USA). To prevent ischemia, a 20 G peripheral catheter was placed as a distal shunt in the artery of each catheterized limb. All intravascular procedures were

performed under fluoroscopic guidance using a C-arm system.

Temperature in the upper and lower half of the body was measured with two sensors placed in the nasopharynx (T_n) and rectum (T_r). In addition, brain, abdominal cavity, and limb temperatures were assessed using radiothermometry (RTM-01-RES, Russia) at a measurement depth of 4–6 cm. Cerebral perfusion quality was monitored using the Invos™ 5100C cerebral/somatic oximeter equipped with Somasensor technology (Medtronic, USA). This device uses near-infrared spectroscopy to determine tissue oxygen saturation (StO_2). Intracranial pressure (ICP) was monitored through a trepanation hole in the left hemicranium, where a parenchymal sensor was placed prior to systemic heparinization (Spiegelberg, Germany). A catheter was placed in the bladder to monitor urine output.

Prior to cannulation, systemic heparinization was performed by administering 70–90 IU of heparin per kilogram of body weight (Heparin Sodium Braun, B. Braun, Germany) to achieve an activated clotting time (ACT) of at least 350 seconds (Actalyke Mini II, Helena Lab., USA).

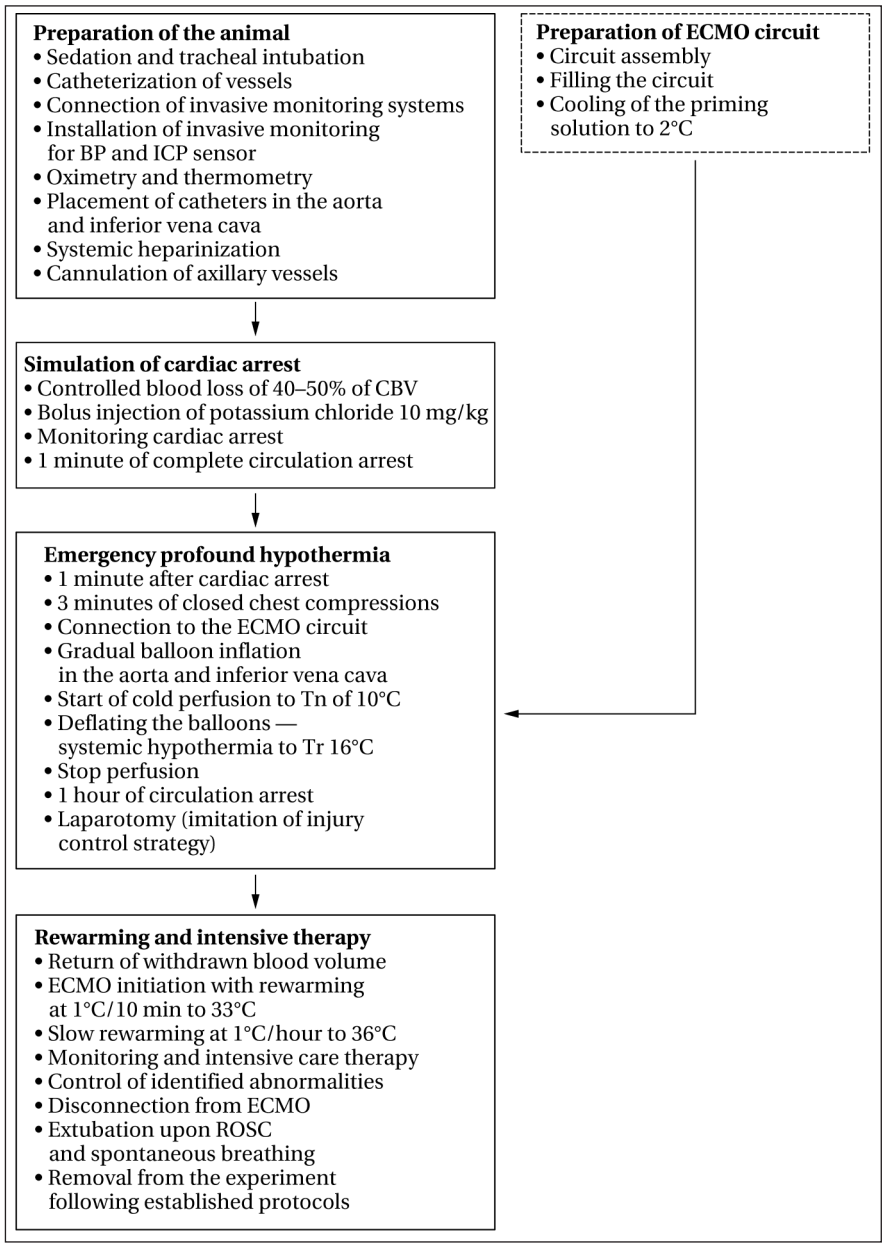

Fig. 1. Experimental protocol for evaluating the efficacy of emergency profound hypothermia in non-human primates.

Note. BP — blood pressure; ROSC — return of spontaneous circulation; ICP — intracranial pressure; CBV — circulating blood volume; T_n — nasopharyngeal temperature; T_r — rectal temperature.

ECMO circuit preparation. The ECMO circuit was preassembled before the procedure and consisted of an RF-36 centrifugal pump head connected to a portable ECMO device (ExStream, Transbiotech, Russia) and an oxygenator (Quadrox-i, Maquet Cardiopulmonary GmbH, Germany). The priming solution for the circuit contained the following components:

— 5 parts Gelofusine (B. Braun, Germany)

— 5 parts Sterofundin (B. Braun, Germany)

— 2 parts 4% sodium bicarbonate solution (Dalchimpharm, Russia)

— 1 part mannitol (Biosynthesis, Russia)

— 1 part 20% albumin (Microgen, Russia)

In addition, 20 mL of 40% glucose and 20 mL of 4% potassium chloride were added to the circuit. The prepared circuit was closed, deaerated, and placed in standby mode for connection. During this time, the prime solution was cooled to 4°C using a thermoregulation unit (TRU, 3T, Sorin Stockert, Germany) connected to the oxygenator.

Simulation of blood loss and cardiac arrest. Controlled blood loss of 40–50% of the circulating blood volume (CBV) was simulated by slowly (over 30–45 minutes) withdrawing venous blood with a syringe from the femoral vein introducer. Blood was collected in containers containing CPDA-1 anticoagulant for reinfusion during the rewarming phase.

When systolic blood pressure (SBP) dropped below 40 mmHg, blood withdrawal was accelerated

and 4% potassium chloride solution at a dose of 10 mg/kg was administered to induce cardiac arrest. Although not a standard approach, the addition of potassium chloride facilitated rapid cardiac arrest and creation of a timepoint while producing systemic hyperkalemia to prevent early spontaneous rhythm recovery during subsequent rewarming. Cardiac arrest was monitored by ECG, invasive blood pressure measurements, and ultrasound.

Emergency profound hypothermia (EPH). One minute after arrest, chest compressions were initiated and continued for 3 minutes. During this time, the ECMO circuit lines were disconnected and connected to the axillary venous drainage and arterial return cannulae. CPR was then stopped and the EPH resuscitation protocol was initiated. The aortic balloon was inflated, the clamps on the ECMO lines were removed, and perfusion of the upper body with cold prime solution was initiated (Fig. 2, *a*). To accelerate cooling and maintain the target T_n, ice packs were also placed around the head.

After three minutes of cooling, a balloon was inflated in the inferior vena cava (IVC) to isolate the «upper» circulation and accelerate cooling. When the brain temperature reached 10°C, the balloons were deflated and removed, and the clamp on the additional arterial return cannula in the femoral artery was released, initiating perfusion of the lower body (Fig. 2, *b*). External cooling with ice packs placed around the animal's body was added to the invasive cooling.

After cooling the brain to 10°C and the body to 16°C, artificial circulation and mechanical ventilation were suspended for 1 hour. A laparotomy was performed, carefully avoiding damage to internal organs, to simulate a surgical procedure for hemorrhage control. Cooling elements were then placed in the abdominal cavity, which was subsequently closed using a continuous suture.

Rewarming and postoperative monitoring. After 60 minutes, perfusion and mechanical ventilation were resumed, with the animal's previously collected blood added to the ECMO circuit while blood gas abnormalities identified during analysis were corrected. External cooling was discontinued and a relaparotomy was performed to remove the cooling elements. Gradual rewarming was performed with a temperature regulating unit (TRU), maintaining a temperature gradient of no more than 4°C between the circuit and nasopharyngeal temperature (T_n). Rewarming was performed at a rate of 1°C every 10 minutes until 33°C was reached, after which the rate was slowed to 1°C per hour. Once body temperature reached 36°C, passive slow rewarming was

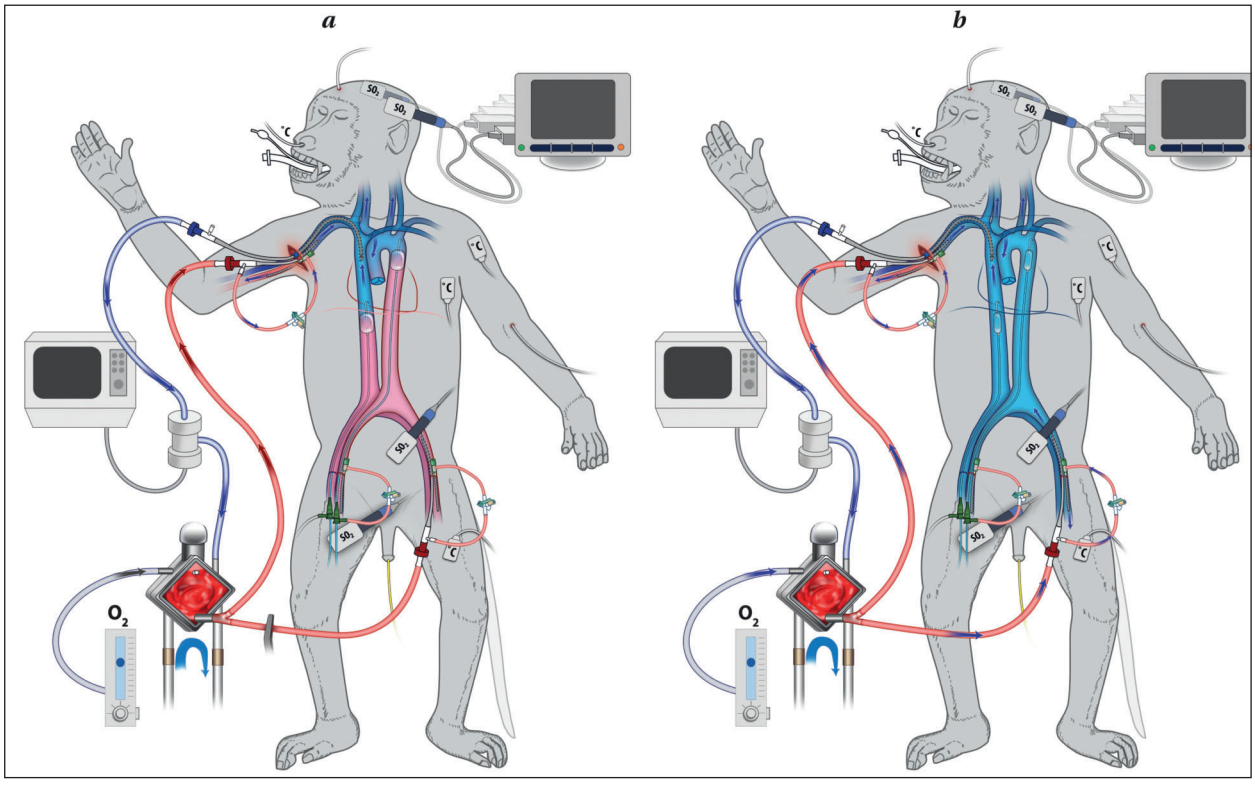

Fig. 2. Graphical representation of the experiment
Note. *a* — initiation of the emergency profound (ultra-deep) hypothermia protocol using a portable ECMO machine. Blood is removed from the superior vena cava and returned to the aortic arch. To isolate the upper circuit for rapid cooling of the brain, a balloon catheter inserted via the femoral artery is inflated at the level of the aortic arch, and an additional balloon is inflated in the inferior vena cava at the level of the caval opening. *b* — both balloons are deflated to allow cooling of the entire body. An additional return cannula in the femoral artery is activated to increase systemic cooling efficiency.

used until normothermia was achieved. The perfusion index was gradually increased from 1.0 (at T_n 10–15°C) to 1.5 (at T_n 15–20°C), 2.0 (at T_n 20–30°C), 2.5 (at T_n 30–35°C), and up to 3.0 at normothermia.

Norepinephrine was administered via an infusion pump to correct systemic hypotension, and adrenaline was added if higher doses were ineffective. Additional infusions of gelofusine, 20% albumin, sodium bicarbonate, and mannitol were given as needed to maintain the calculated flow volume. Furosemide was administered to stimulate diuresis when indicated, and rapid-acting insulin (Actrapid, Novo Nordisk, Denmark) was used to correct hyperglycemia. Sodium heparin was used to prolong the activated clotting time (ACT), and calcium chloride was administered to correct hypocalcemia (serum calcium $<$ 1 mmol/L). If no urine output was observed, an ultrafiltration column (Diacap Pro, B. Braun, Germany) was connected to the ECMO circuit to correct hyperhydration.

In the event of coarse ventricular fibrillation during rewarming, defibrillation was performed by delivering a 100–150 J shock with a Lifepak 12 defibrillator (Medtronic, USA).

Once the T_n reached 37°C and the animal was stabilized with a sustained spontaneous breathing pattern and limb movement, endotracheal extubation was performed. The animal was then returned to the vivarium for further observation. In the absence of spontaneous cardiac activity and respiration after complete rewarming, biological death was confirmed, and the animal was euthanized by exsanguination under general anesthesia.

The primary endpoint was return of spontaneous circulation (ROSC), while secondary endpoints included 24-hour survival and recovery from anesthesia.

Blood biochemical parameters were measured spectrophotometrically using a Chemray 240 automated analyzer (Rayto, China). Lactate levels were assessed by direct amperometric analysis using a GemPremier 3500 (IL Werfen, USA), which was also used for blood gas composition and acid-base status analysis.

Tissue samples for histologic analysis were fixed in 10% neutral buffered formalin (pH 7.4). Standard histologic processing was performed followed by paraffin embedding (Histomix, BioVitrum LLC, St. Petersburg, Russia). Paraffin blocks were cut at 4 μm and stained with hematoxylin and eosin. Morphological analysis was performed with an Axio Lab.A1 biological microscope (Carl Zeiss Microscopy GmbH, Germany), and microphotographs were taken with an Axiocam 105 color digital camera (Carl Zeiss Microscopy GmbH, Germany).

The identified changes in tissues and internal organs at both macroscopic and microscopic levels were thoroughly described and documented in the protocols of pathological anatomical examination.

Data collection and registration were performed using Excel (Microsoft, USA). Statistical analysis and graphing were performed using Prism 10.0 (GraphPad, USA). Due to the lack of a comparison group, no comparative analysis of the samples was performed. Quantitative variables (including those in Fig. 3) were presented as median with interquartile range.

Results and Discussion

In all five cases, the proposed TCA model was successfully implemented, achieving a brain temperature of 10°C and a body temperature of 16°C. During controlled blood loss, 600 (400–760) ml of blood was collected and prepared for subsequent reinfusion. The mean time for brain cooling was 23 (9–36) minutes. The maximum possible time to cool the brain to 10°C was 7 minutes. The variability in cooling rate was directly related to the flow in the ECMO circuit. When the walls of the vein collapsed during blood withdrawal from the vena cava superior, the flow decreased, which slowed the entire cooling process.

During the gradual rewarming, the T_n and T_r gradually converged and began to rise (Fig. 3, *a*). When the nasopharyngeal temperature reached 18°C, spontaneous electrical activity of the heart was observed (initially, the rhythm was not sinus). When the temperature reached 22–25°C, the first spontaneous cardiac contractions occurred, often progressing to ventricular fibrillation (VF). Considering VF to be potentially damaging to the myocardium, these episodes were managed by administering additional potassium chloride. Sinus rhythm was restored when the T_n reached approximately 28–30°C. In two cases, this required 1–2 defibrillator shocks at 120–150 J. In one of the five animals (No. 1), sustained ROSC was not achieved despite the appearance of rhythm and spontaneous cardiac contractions, and the experiment was terminated prematurely after 13 hours of rewarming. Thus, ROSC was successfully achieved in 4 out of 5 animals (Table).

Cerebral and cardiac perfusion. Despite induction of profound hypothermia and circulatory arrest at a brain temperature of 10°C, no decrease in cerebral oximetry values was observed (Fig. 3, *b*).

During the 12 hours of rewarming, cerebral oximetry values remained consistently above the threshold of 40%, indicating adequate cerebral perfusion. Later, however, with progressive hypotension poorly controlled by vasopressors, oximetry values dropped to 20% and below. At the same time, intracranial pressure (ICP) remained within acceptable limits in 4 out of 5 animals. In animal No. 3, ICP increased after 16 hours of the experiment, followed by death (Table).

Systemic coagulopathy and ischemia-reperfusion injury. When the T_n reached 26–30°C, 3 of the 5 animals developed severe coagulopathy char-

Table. Summary of data on experimental animals, treatment outcomes, and causes of death.

Parameter	The values of the parameters for each individual olive baboon (*Papio anubis*)				
	1	**2**	**3**	**4**	**5**
Sex	male	male	male	male	male
Body weight, kg	19.8	19.6	18.0	21.0	26.7
Blood loss, mL	820	500	300	700	600
Time to cooling to 10°C, min	35	7	37	23	11
ROSC	—	+	+	+	+
ECMO weaning	—	+	—	—	+
Extubation	—	+	—	—	+
Survival time from the start of the experiment, hours	15	44	16	19	19
Main pathological findings	Pulmonary edema, multiple organ failure	Myocardial infarction	Pulmonary edema, cerebral edema	Multiple organ failure, small intestine ischemia, abdominal compartment syndrome	Pulmonary edema

Note. ROSC — return of spontaneous circulation; ECMO — extracorporeal membrane oxygenation.

acterized by bleeding from surgical access sites and diffuse intra-abdominal hemorrhage. These complications required surgical wound revision, use of electrocautery, application of local hemostatic agents, tight wound packing, and intravenous administration of 1.0 g tranexamic acid. Moderate acceleration of the rewarming process at these temperatures was found to help bypass the coagulopathy phase more quickly, reducing the risk of fatal blood loss.

In one animal (No. 2), efforts to control bleeding from a wound in the right axillary region resulted in inadvertent decannulation, causing massive hemorrhage. The bleeding was promptly controlled by clamping the vessel and ligating the axillary artery. Mechanical circulatory support was maintained exclusively through the femoral arterial cannula, with no evidence of upper limb ischemia. The animal was later successfully extubated.

During the first 5–6 hours of rewarming, lactate levels remained elevated in all animals (approximately 10 mmol/L), but could be managed by increasing the perfusion index, transfusing blood, and, if necessary, removing excess fluid from the circulating blood. However, despite intensive therapy, lactate levels subsequently rose rapidly to the maximum measurable by the device (15 mmol/L). In animals that later succumbed, lactate concentrations exceeded 15 mmol/L from the 8th hour of rewarming. In contrast, surviving animals showed successful control of progressive lactic acidosis. Systemic reperfusion was identified as the most significant factor contributing to mortality (Fig. 3, *b*).

Blood biochemistry showed an increase in transaminase activity: AST increased from 55.4 (46.8–64.0) to 171.4 (150.4–192.3) and then to 301.3 (239.9–362.6) U/L, while ALT increased from 14.5 (5.8–23.1) to 20.9 (17.8–24.0) and then to 48.3 (16.3–80.3) U/L at 5 and 7 hours after the start of rewarming. In addition, creatinine concentrations increased from 149 (145–152) to 155 (115–196) and then to 190 (135–245) μmol/L at 7 and 9 hours, respectively, reflecting systemic hypoperfusion and the development of multiorgan failure. However, these parameters were successfully corrected in surviving animals by intensive therapeutic measures (Fig. 4, *a*, *b*, *c*).

A significant increase in creatine phosphokinase (CPK) activity was also observed, rising from 202 (200–205) to 1105 (1042–1167) and subsequently to 2045 (1710–2380) U/L at 5 and 7 hours after rewarming, respectively (Fig. 4, *d*). In animals that did not survive, CPK levels remained elevated, whereas in survivors, these levels returned closer to baseline levels.

Survival. Endotracheal extubation was successful in 2 of 5 animals. Their cooling times were 7 and 11 minutes, respectively. One animal underwent extubation after cannula removal 16 hours after the start of the experiment. Although it was returned to the vivarium, the animal died 44 hours into the experiment without regaining full consciousness. This precluded assessment of brain function. Autopsy revealed ischemic myocardial damage and moderate cerebral edema.

Another animal survived the procedure with successful tracheal extubation. However, while being moved to the vivarium, it developed respiratory failure, probably due to respiratory muscle fatigue, and subsequently died. Postmortem examination revealed severe pulmonary edema (Fig. 5).

Both «surviving» animals showed purposeful motor activity, including fist clenching, spontaneous eye opening, and mouth movements.

Three of the five animals died on the operating table 14.5 to 18.5 hours after the start of rewarming. It took 23, 35, and 37 minutes, respectively, to reach the target T_n. According to postmortem findings, the primary causes of death were pulmonary edema and, in some cases, ischemic liver injury.

Animal No.4 developed abdominal compartment syndrome (ACS) due to excessive fluid therapy, requiring

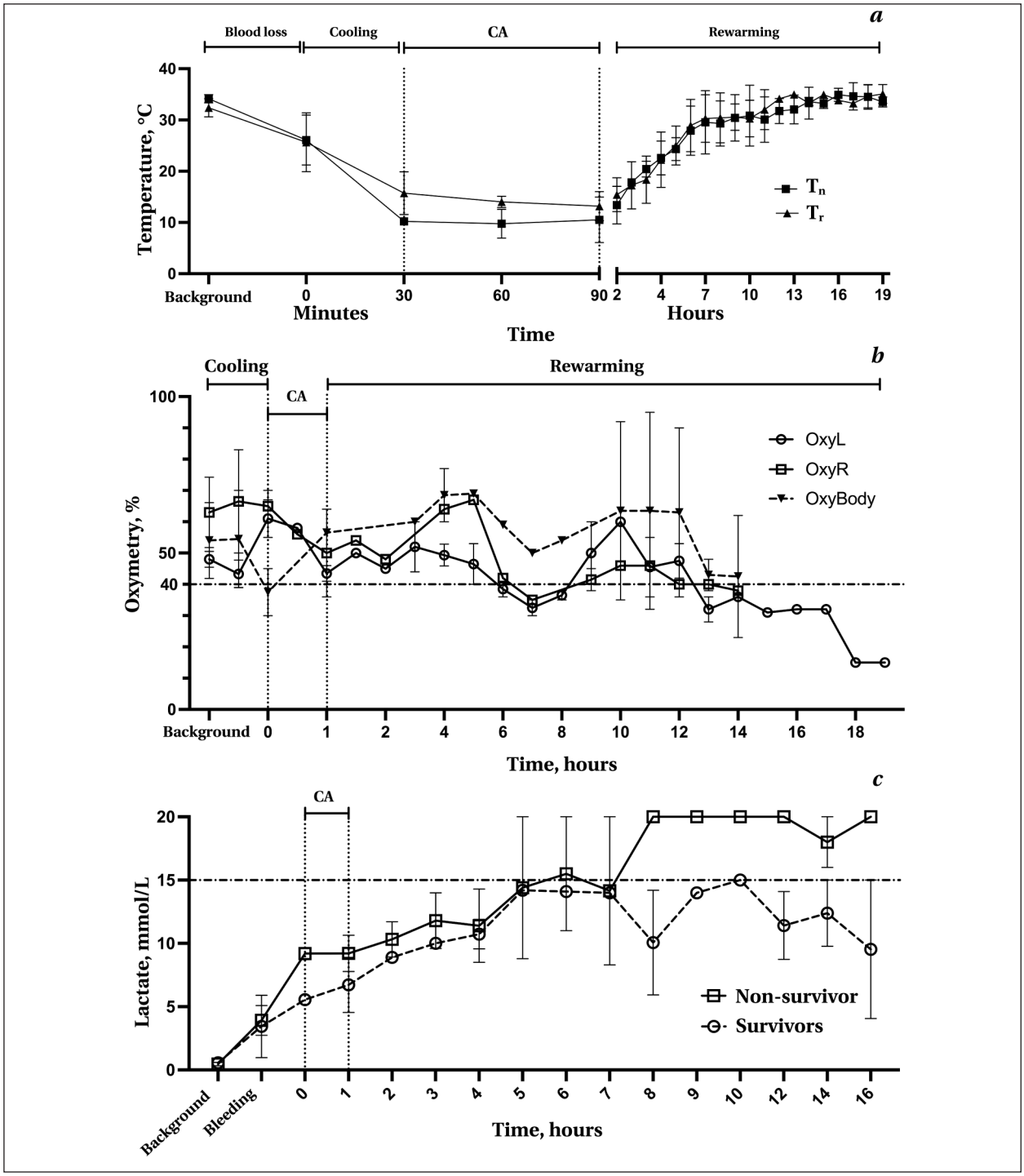

Fig. 3. Changes in key parameters monitored during the experiment.
Note. *a* — nasopharyngeal (Tn) and rectal (Tr) temperatures; *b* — cerebral oximetry in the left and right hemispheres (OxyL and OxyR) and tissue oximetry in the lumbar region (OxyBody); *c* — serum lactate concentrations, stratified by surviving (*N*=2) and non-surviving (*N*=3) animals. The horizontal line at 15 mmol/L indicates the threshold measurable by the blood gas analyzer. Values above this line, shown at the 20 mmol/L level, are presented for illustrative purposes and were excluded from the calculations. CA — circulatory arrest.

a relaparotomy and laparostomy (Bogotá bag). This animal also had progressively increasing ICP during rewarming, reaching 47 mmHg, which was refractory to conservative management and remained elevated after resolution of the ACS. Postmortem examination revealed cerebral edema in addition to total ischemic damage to the small intestine.

Histologic examination. All animals showed signs of cerebral edema of varying severity, dystrophic changes in neurons (cytoplasmic vacuolization, nuclear deformation), and plasmatic imbibition of vascular walls (Fig. 6, *a*). Myocardial changes were also observed in all cases, ranging from diapedetic microhemorrhages, edema, and fragmentation of

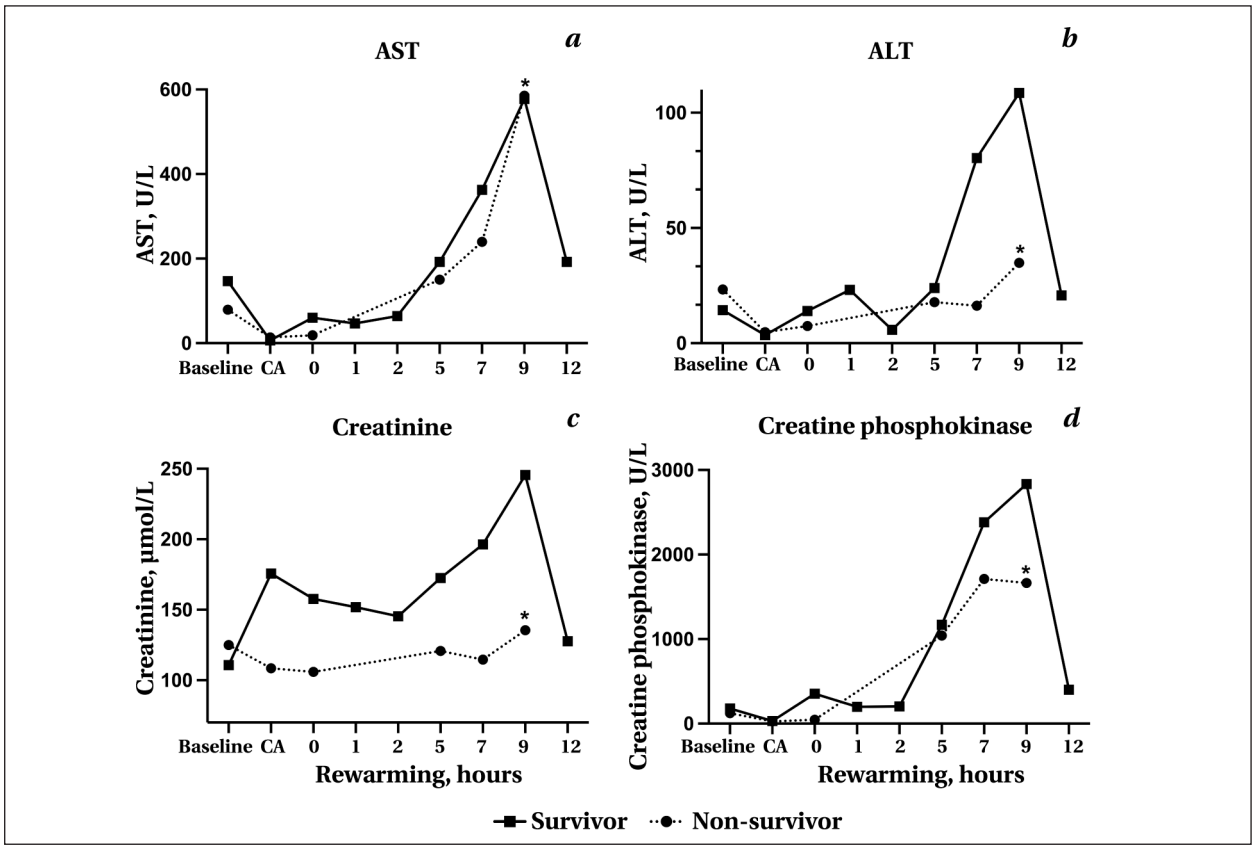

Fig. 4. Trends in key biochemical parameters in representative animals: one surviving and one non-surviving.
Note. *a* — AST; *b* — ALT; *c* — creatinine; *d* — creatine phosphokinase. * — final time point of biochemical analysis for the non-survivor; CA — circulatory arrest.

individual muscle fibers to intramural necrosis. Pulmonary findings included acute alveolar emphysema, focal dystelectasis, congestion, and inflammatory infiltration.

In the animal that died 44 hours into the experiment, cerebral edema was accompanied by extensive non-coronary myocardial necrosis of the left ventricle and alveolar pulmonary edema (Fig. 6, *b*). The animal with ACS demonstrated necrosis of the intestinal wall and hydropic degeneration of hepatocytes (Fig. 6, *c*). Renal histopathology was mild and included exudative glomerulopathy, tubular and interstitial edema, and tubular epithelial degeneration (Fig. 6, *d*).

Thus, this study demonstrated the feasibility of using the EPH technique in TCA. Target brain (10°C) and body (16°C) temperatures were successfully achieved in all animals within a short time, ensuring adequate cerebral oxygenation, allowing for surgical intervention and further controlled rewarming of the body. Return of spontaneous circulation was achieved in 4 out of 5 animals, and 2 animals were successfully weaned from ECMO and extubated. However, both animals died in the early postoperative period, precluding evaluation of cognitive function. The main causes of mortality during the rewarming period were ischemia-reperfusion injury, cerebral edema and transient coagulopathy.

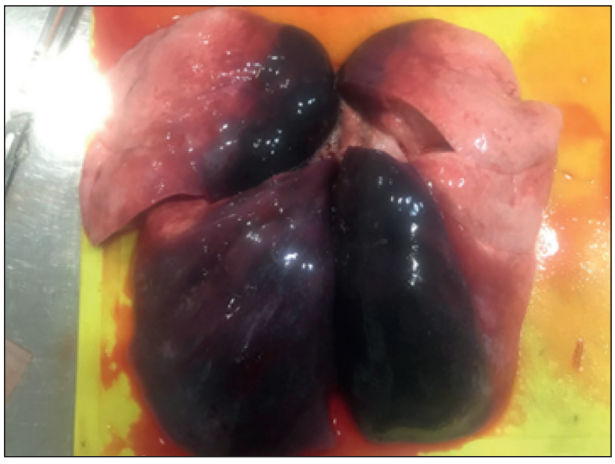

Fig. 5. Postmortem photograph of the lungs of one of the animals (No. 5) that died after tracheal extubation due to progressive respiratory failure and development of acute respiratory distress syndrome (ARDS).

Prolonged blood collection leading to systemic hypoxemia and hypoperfusion, the inability to achieve rapid cooling in all cases, and a period of no-flow in the vessels of the lower half of the body during cooling of the upper half (T_n reached 10°C while T_r remained at 35°C) resulted in severe systemic ischemia. This ischemia caused an accumulation

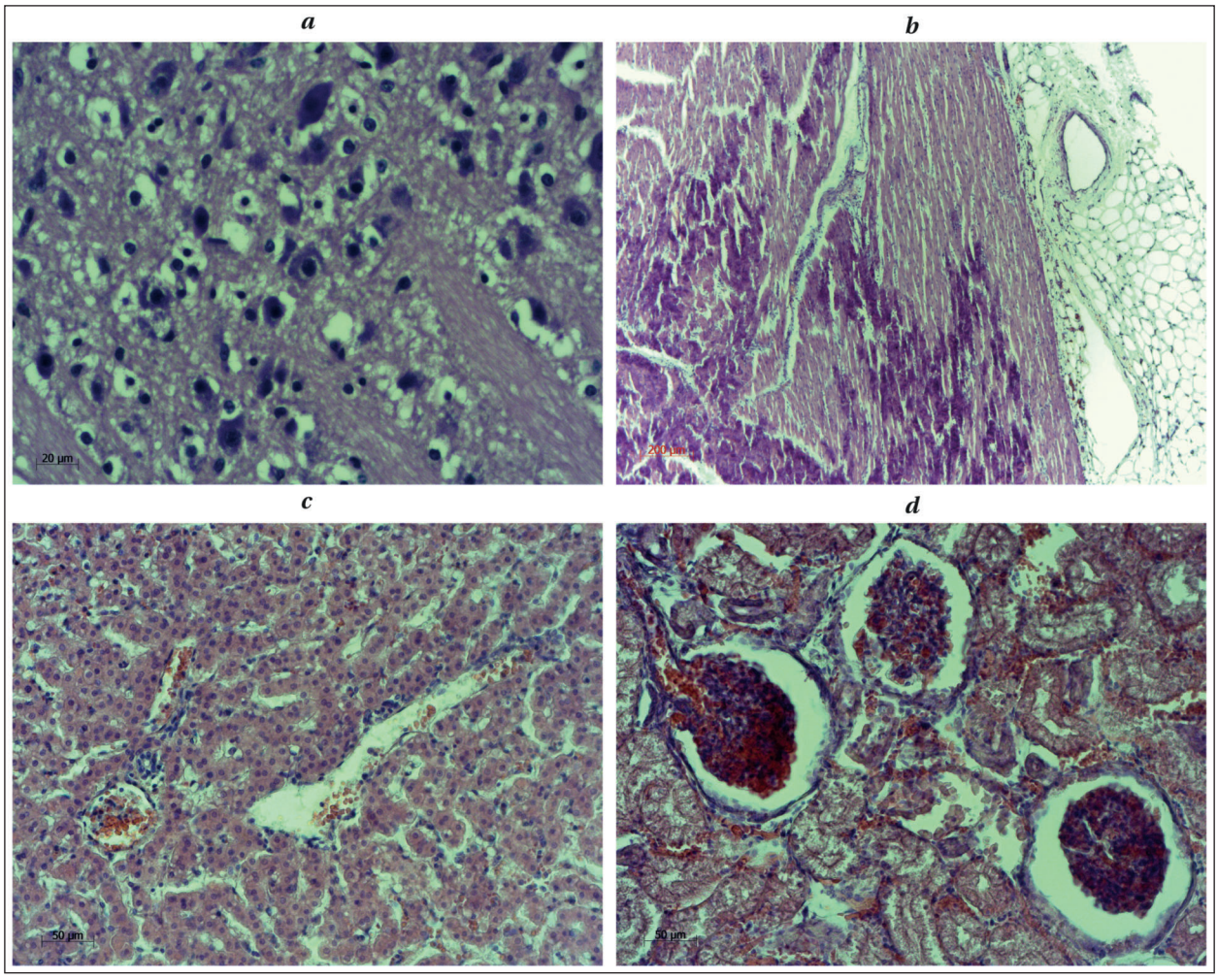

Fig. 6. Representative microstructural images of vital organs from animals subjected to emergency profound (ultra-deep) hypothermia.
Note. a — Thalamus: cerebral edema, plasmatic imbibition of vascular walls, dystrophic neuronal changes (cytoplasmic vacuolization, nuclear deformation) (hematoxylin-eosin stain, ×400). *b* — Lateral wall of left ventricular myocardium: intramural myocardial necrosis (dark purple staining, hematoxylin-eosin, ×50). *c* — Liver: perisinusoidal edema, severe hydropic degeneration of hepatocytes (hematoxylin-eosin, ×200). *d* — Kidney: exudative glomerulopathy, tubular and interstitial edema, dilatation of glomerular capsules, proliferation of parietal epithelial cells and hydropic degeneration of tubular epithelium (hematoxylin-eosin, ×200).

of under-oxidized metabolites in the internal organs and muscles of the lower extremities. Subsequently, despite lowering the T_r to target levels, rewarming was complicated by rhabdomyolysis (confirmed by a significant increase in creatine phosphokinase activity) and severe reperfusion injury, which could not be alleviated by slow temperature rise.

Studies by others have shown that to prevent severe reperfusion injury, cooling must be as rapid as possible while rewarming must be slow. An optimal cooling rate of 2°C/min was demonstrated by H. Alam et al. in porcine models [14]. This was achieved by open cannulation of the aorta and right atrium with large diameter cannulae after resuscitative thoracotomy. However, too rapid cooling carries the risk of cold-induced damage to vital organs, while too slow cooling increases the likelihood of ischemia-reperfusion syndrome.

Therefore, T_n should ideally be reduced to 26°C within approximately 13 minutes. This was achieved only twice in the current study, specifically in the two animals classified as «survivors» (7–11 minutes). However, in the animal that survived 44 hours after the procedure and reached the target T_n in 7 minutes, postmortem examination revealed a massive myocardial infarction, which could be partially attributed to cold-induced injury.

The progression of ischemia-reperfusion syndrome is evidenced by a gradual increase in serum lactate concentration (> 15 mmol/L), likely due to a combination of factors: prolonged exsanguination, one minute of normothermic circulatory arrest, three minutes of chest compressions (generally ineffective in the setting of massive blood loss), prolonged lower body ischemia during upper body cooling, and 60 minutes of hypothermic circulatory

arrest. Notably, as several studies have shown, hypothermic circulatory arrest of this duration is not inherently a cause of irreversible cellular or organelle damage, as evidenced by survival rates exceeding 50% with favorable functional outcomes [17, 18].

The observed uncontrolled rise in lactate concentration during the early phases of reperfusion likely serves as a prognostic marker for adverse outcomes [19]. Similar lactate levels were recorded in a previous experiment using EPH during simulated tactical military medical exercises [20]. Large pigs with abdominal gunshot wounds underwent EPH and were surgically treated in a reinforced concrete bunker simulating a forward surgical care environment. They were transported 50 km in a hypothermic state, without mechanical ventilation or circulatory support, to a simulated advanced surgical care facility where rewarming was initiated. It took over an hour to reach the target T_n. Although ROSC was achieved after two hours of transport, the animals ultimately succumbed to severe reperfusion injury [20].

Another critical issue affecting survival was cerebral edema, which was observed in all animals. This was primarily related to inadequate replacement of blood loss with crystalloids and colloids. Despite monitoring of ICP and cerebral oximetry, as well as pharmacologic and non-pharmacologic intensive care, it was not possible to completely resolve this complication. The death of the only long-lived animal was primarily due to progressive cerebral edema, as confirmed by autopsy findings. During the experiment, computed tomography imaging was not available to assess brain damage.

Finally, one of the life-threatening problems was coagulopathy, which consistently manifested itself when temperatures reached 27–32°C during rewarming. This was characterized by diffuse bleeding from existing wounds and access sites (including puncture sites), requiring both surgical and pharmacologic hemostasis. As a result, the volumetric flow in the ECMO circuit decreased, requiring additional fluid infusion and subsequently provoking ischemia-reperfusion. Intravenous administration of tranexamic acid was found to have little effect on the intensity of bleeding. S. Tisherman and colleagues also highlighted the challenges of managing coagulopathic bleeding during the rewarming phase and addressed this by increasing the rewarming rate until bleeding stopped [15].

Therefore, slow rewarming is necessary to avoid severe reperfusion injury and edema; however, too slow rewarming results in coagulopathic bleeding, which can be fatal with extensive tissue damage. Similarly, cooling must be as rapid as possible, but too rapid cooling can cause cold injury to vital organs.The optimal cooling and rewarming rates require further investigation. Our experimental data indicate that the best results are achieved with cooling over 10–15 minutes and rewarming over at least 12–16 hours.

Encouraging results from numerous U.S. research groups demonstrating sufficient animal survival after two hours of circulatory arrest with EPH led the FDA to approve a pilot randomized controlled clinical trial (EPR-CAT) enrolling 10 patients in an EPH group and 10 in a control group. Due to the COVID-19 pandemic, the trial was temporarily suspended and the results remain unpublished. Furthermore, to our knowledge (personal communication with S. Tisherman), there have been no successful human cases. Nevertheless, the FDA's approval of this trial highlights the significant interest in hypothermia for terminal conditions and novel approaches to saving civilian and military casualties.

Our study has several limitations that may have significantly influenced the results. First, the EPH procedure is resource-intensive, requiring high-quality (around-the-clock) monitoring, intensive care, and extensive use of blood and blood products, which is difficult to achieve in an experimental operating room. At a minimum, animals should have undergone computed tomography (CT) or magnetic resonance imaging (MRI) of the brain, electroencephalography, and coagulation studies after extubation; these were not available at the time. While blood and blood product replacement is preferable to crystalloid solutions for volume resuscitation, donor blood components are rarely available in animal studies and virtually never in nonhuman primate studies.

Second, the cooling rate was relatively slow for both the upper and lower body. Achieving target temperatures more rapidly would have required larger diameter cannulas than the 15–17 Fr cannulas we used. In animals weighing approximately 20 kg, larger cannulas would require open aortic and vena cava cannulation, which carries additional risks of surgical trauma and bleeding.

Finally, the small number of animals and the lack of a control group are significant limitations due to the pilot nature of this study.

Nevertheless, the results of this first-of-its-kind experiment in non-human primates underscore the importance of pursuing novel solutions to help those previously considered beyond saving. The concept of using EPH, developed by our esteemed compatriot V. A. Negovsky and his successors, including P. Safar and his student S. Tisherman, is both technically and physiologically feasible in the context of terminal states. Rapid cooling of the brain, heart, and, if necessary, the entire organism during TCA allows a 1–2-hour window for life-saving surgery, blood product procurement, and transport to a specialized center where gradual rewarming can restore vital functions. Demonstrating the effi-

cacy of this method will open new avenues for exploring various applications of rapid whole-body external cooling, allowing temporary preservation not only of organs and tissues, but of the entire organism, followed by rewarming after life-threatening injuries have been treated.

Conclusion

The experiment showed that emergency profound (ultra-deep) hypothermia, with head cooling to 10°C and core cooling to 16°C, preserves adequate perfusion of vital organs and tissues despite 60 minutes of complete circulatory arrest. In addition, faster cooling rates are associated with better chances of recovery and survival. During rewarming, cardiac function is restored and successful management of ischemia-reperfusion syndrome, vital organ edema and coagulopathy leads to resuscitation. Further research, including studies in other animal models, is needed to better understand the causes of serious complications and to refine the EPH protocol.

References

1. *Konesky K. L., Guo W. A.* Revisiting traumatic cardiac arrest: should CPR be initiated? *Eur J Trauma Emerg Surg.* 2018; 44 (6): 903. DOI: 10.1007/s00068-017-0875-6. PMID: 29177620.
2. *Gräsner J. T., Wnent J., Herlitz J., Perkins G. D., Lefering R., Tjelmeland I., Koster R. W., et al.* Survival after out-of-hospital cardiac arrest in Europe — Results of the EuReCa TWO study. *Resuscitation.* 2020; 148: 218–226. DOI: 10.1016/j.resuscitation.2019.12.042. PMID: 32027980.
3. *Rhee P. M., Acosta J., Bridgeman A., Wang D., Jordan M., Rich N.* Survival after emergency department thoracotomy: review of published data from the past 25 years. *J Am Coll Surg.* 2000; 190 (3): 288–298. DOI: 10.1016/s1072-7515(99)00233-1. PMID: 10703853.
4. *Beske R. P., Obling L., Bro-Jeppesen J., Nielsen N., Meyer M., Kjaergaard J., Johansson P. I., et al.* The effect of targeted temperature management on the metabolome following out-of-hospital cardiac arrest. *Ther Hypothermia Temp Manag.* 2023; 13 (4): 208–215. DOI: 10.1089/ther.2022.0065. PMID: 37219970.
5. *Неговский В. А.* Оживление организма и искусственная гипотермия. М.: Медгиз; 1960: 302. *Negovsky V. A.* The resuscitation of the body and artificial hypothermia. M.: Medgiz; 1960: 302.
6. *Marchand P., Allan J. C.* An experimental study of the effect of hypothermia on the heart and brain. *S Afr J Med Sci.* 1956; 21 (3–4): 127–141. DOI: 10.1097/00132586-195810000-00002. PMID: 13442723.
7. *Bigelow W. G., Lindsay W. K., Greenwood W. F.* Hypothermia; its possible role in cardiac surgery: an investigation of factors governing survival in dogs at low body temperatures. *Ann Surg.* 1950; 132 (5): 849–866. DOI: 10.1097/00000658-195011000-00001. PMID: 14771796.
8. *Мороз В. В.* К 100-летию академика РАМН Владимира Александровича Неговского. *Общая реаниматология.* 2009; 5 (1): 3–11. *Moroz V. V.* On the occasion of the100th anniversary of V. A. Negovsky academician of the Russian Academy of Medical Sciences. *General Reanimatology = Obshchaya Reanimatologiya.* 2009; 5 (1): 3–11. (In Russ.&Eng.). DOI: 10.15360/1813-9779-2009-1-5.
9. *Chamberlain D.* «Vladimar Negovsky: The Father of reanimatology». The Negovsky memorial lecture, given at the seventh scientific congress of the European Resuscitation Council, Budapest September 2004. *Общая реаниматология. General Reanimatology = Obshchaya Reanimatologiya.* 2005; 1 (2): 57–69. DOI: 10.15360/1813-9779-2005-2-57-69.
10. *Safar P. J., Tisherman S. A.* Suspended animation for delayed resuscitation. *Curr Opin Anaesthesiol.* 2002; 15 (2): 203–210. DOI: 10.1097/00001503-200204000-00010. PMID: 17019202.
11. *Wu X., Drabek T., Tisherman S. A., Henchir J., Stezoski S. W., Culver S., Kochanek P. M.* Emergency preservation and resuscitation with profound hypothermia, oxygen, and glucose allows reliable neurological recovery after 3 h of cardiac arrest from rapid exsanguination in dogs. *J Cereb Blood Flow Metab.* 2008; 28 (2): 302–311. DOI: 10.1038/sj.jcbfm.9600524. PMID: 17622254.
12. *Усенко Л. В., Царев А. В.* Искусственная гипотермия в современной реаниматологии. *Общая реаниматология.* 2009; 5 (1): 21–23. *Usenko L. V., Tsarev A. V.* Artificial hypothermia in modern reanimaology. *General Reanimatology = Obshchaya Reanimatologiya.* 2009; 5 (1): 21–23. (in Russ.&Eng.). DOI: 10.15360/1813-9779-2009-1-21.
13. *Capone A., Safar P., Radovsky A., Wang Y. F., Peitzman A., Tisherman S. A.* Complete recovery after normothermic hemorrhagic shock and profound hypothermic circulatory arrest of 60 minutes in dogs. *J Trauma.* 1996; 40 (3): 388–395. DOI: 10.1097/00005373-199603000-00011. PMID: 8601855.
14. *Alam H. B., Chen Z., Honma K., Koustova E., Querol R. I. L. C., Jaskille A., Inocencio R., et al.* The rate of induction of hypothermic arrest determines the outcome in a swine model of lethal hemorrhage. *J Trauma.* 2004; 57 (5): 961–969. DOI: 10.1097/01.ta.0000149549.72389.3f. PMID: 15580018.
15. *Tisherman S. A.* Emergency preservation and resuscitation for cardiac arrest from trauma. *Ann N Y Acad Sci.* 2022; 1509 (1): 5–11. DOI: 10.1111/nyas.14725. PMID: 34859446.
16. *Castellini G., Gianola S., Biffi A.* Resuscitative endovascular balloon occlusion of the aorta (REBOA) in patients with major trauma and uncontrolled haemorrhagic shock: a systematic review with meta-analysis. *World J Emerg Surg.* 2021; 16 (1): 41. DOI: 10.1186/s13017-021-00386-9. PMID: 34384452.
17. *Alam H. B., Rhee P., Honma K., Chen H., Ayuste E. C., Lin T., Toruno K., et al.* Does the rate of rewarming from profound hypothermic arrest influence the outcome in a swine model of lethal hemorrhage? *J Trauma.* 2006; 60 (1): 134–146. DOI: 10.1097/01.ta.0000198469.95292.ec. PMID: 16456447.
18. *Liu Y., Li S., Zhang J., Han J., Zhang Y., Yin Z., Wang H.* A safety evaluation of profound hy-

pothermia-induced suspended animation for delayed resuscitation at 90 or 120 min. *Mil Med Res*. 2017; 4: 16.
DOI: 10.1186/s40779-017-0127-4.
PMID: 28573043.

19. *Thevathasan T., Gregers E., Mørk S. R., Degbeon S., Linde L., Andreasen J. B., Smerup M., et al.* Lactate and lactate clearance as predictors of one-year survival in extracorporeal cardiopulmonary resuscitation — An international, multicentre cohort study. *J Resuscitation*. 2024; 198: 110149.
DOI: 10.1016/j.resuscitation.2024.110149.
PMID: 38403182.
20. *Рева В. А., Гончаров С. Ф., Потемкин В. Д., Баранов М. И., Вертий А. Б., Сажнева М. Ю., Самакаева А. Р., с соавт.* Экстренная сверхглубокая гипотермия при остановке кровообращения, вызванной массивной кровопотерей: из опыта проведения тактико-специальных учений. *Медицина катастроф*. 2023; 4: 57–64. *Reva V. A., Goncharov S. F., Potemkin V. D., Baranov M. I., Vertiy A. B., Sazhneva M. Y., Samakaeva A. R., et al.* Emergency superdeep hypothermia in circulatory arrest caused by massive blood loss: from the experience of tactical and special exercises. *Disaster Medicine = Meditsina Katastrof*. 2023; 4: 57–64. (in Russ.).
DOI: 10.33266/2070-1004-2023-4.

Received 20.08.2024
Accepted 11.12.2024

Index of Authors and Papers Published in the Journal «General Reanimatology» in 2024*

* The Number before the Bracket is the Number of the Journal; the Numbers in the Brackets are the Pages of the Paper.

Instructions for Authors of the General Reanimatology Journal

Based on the «Brief author guidelines for preparing and formatting scholarly papers in journals indexed in international scientific databases'edited by Olga Kirillova under the ASEP (Association of Scientific Editors and Publishers) and RRIEPL (Russian Research Institute of Economics, Politics and Law in Science and Technology) published in 2019, the CSE's White Paper on Promoting Integrity in Scientific Journal Publications, 2012 Update, **ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (December 2016)**, and the European Association of Scientific Editors (EASE) Guidelines for Authors and Translators (available at **https://ease.org.uk/guidelines-toolkits/**).

Version Dated February 2023

When submitting a manuscript to the General Reanimatology journal, the authors guarantee that:

— the manuscript has not been previously published in another journal;

— the manuscript is not currently reviewed for publication in another journal;

— the manuscript does not contain any confidential information;

— all co-authors agree with publication of the current version of the article.

Instructions for the Authors Before Submitting the Manuscript

Before submitting a manuscript for review, make sure that the file contains all the necessary information in Russian or English, lists all sources of information (references), has a full set of figures and tables, all citations are properly formatted.

The editorial board of the «General Resuscitation» journal recommends that authors use the following checklists and charts developed by international health organizations in preparing manuscripts and other materials (**EQUATOR, Enhancing the Quality and Transparency of Health Research, https://www.equator-network.org/reporting-guidelines/; SWIHM, Scientific Writing in Health & Medicine https://www.swihm.com/course/**):

When preparing papers reporting the results of randomized clinical trials, **«CONSORT 2010 checklist of information to include when reporting a randomized trial»**, **https://www.equator-network.org/reporting-guidelines/consort/**, should be used.

When preparing papers reporting the results of non-experimental research, **«The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies»**, **https://www.equator-network.org/reporting-guidelines/strobe/**, should be used.

When preparing a systematic review, **«PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)»**, **https://www.equator-network.org/reporting-guidelines/prisma/**, should be used. Additionally, we recommend the following outline for the abstract (summary): scope of the problem (1–3 sentences from the introduction); aim of the review (the same wording in the summary and in the introduction); number of sources, criteria and databases of source selection; specific issues considered according to the highlighted subheadings in the body of the review); limitations of the research on the topic; conclusion (an abridged version of the conclusion from the body of the review).

When preparing a clinical case report/series, **«The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development»**, **https://www.care-statement.org/checklist/**, or **SWIHM** 2019 recommendations should be used. Russian language form can be found at **www.reanimatology.com** → Section «Authors Guidelines» → Case Report Writing Template for Authors.

When preparing papers reporting the results of qualitative research, **SRQR (Standards for reporting qualitative research)**, **https://www.equator-network.org/reporting-guidelines/srqr/**, should be used.

Full version at www.reanimatology.com

Main information for the manuscript submission

PARAMETER	INSTRUCTIONS
Limitations	
Initial submission	One file in the Word format in Russian for Russian-speaking authors in English for non-Russian-speaking authors, including: — the title of the paper; — full names of all authors; — affiliations of all authors; — IDs of profiles in the scientific databases for each author; — the text of all sections of the paper; — tables, figures, photos with captions and notes; — references; — conflict of interest; — information of study funding; — acknowledgements (optional); — authors' contribution (preferably)
The length of the manuscript	Original manuscript — about 40,000 characters with spaces; Short communication — should not exceed 2,500 words; Review, meta-analysis — 25,000–40,000 characters with spaces
Front page information	
Title of the paper	Should not exceed 15 words
Information about authors	Full name (Peter A. Johnson), author profile ID in the research database(s) for each author (e-Library/RSCI (Rus), ORCID, Scopus, WoS researcher ID if available)
Affiliations	Full name and postal address of the organizations with zip code
Corresponding author	Full name, e-mail address, phone number
The paper outline and references	
Summary (abstract)	250–300 words. Sections: scope of the problem (introduction/background), aim, material and methods, results, conclusion
Highlights (main messages as text or infographics, an optional section following the summary)	1–3 messages in graphic or text form (no more than 40 words per each text message)
Key words	6–8 words listed with a semicolon (;), without a dot at the end
Body of the paper	Sections: introduction (background), material and methods, results, discussion, conclusion
Supplementary information sections	Conflict of interest, funding of the study should follow the Keywords paragraph. Acknowledgements (optional) and authors' contribution (preferably) should be placed at the end of the paper
Illustrations, including tables	Original paper — up to 8; Short communication — no more than 3; Review — up to 8
References	Dating: 70% should be published within the last 5 years, of them at least 30% within the last 3 years. Number: Original paper — 25–45; Short communication — 10–25; Review — 80–120. Format: please see the «References Formatting» section, www.reanimatology.com
Formatting	
Font	Times New Roman, 12 points. The section titles should be typed in bold
Spacing and Indentation	Line spacing — 1.5; Interval before and after the paragraph — none; Interval between sections — one extra spacing; First line indent — 1.25 cm
Fields	2.5 cm on all sides
Page numbering	In the lower right corner

Dear Colleagues,

We invite you to participate
in the 4th Scientific and Educational Conference

Medicine and Innovation-2025: From Fundamental Science to Hybrid Biotechnologies in Clinical Practice

March 3–4, 2025
10:00 – 17:00 (Moscow Time, MSK)

Information about the scientific organizers
and topics of presentations is available on the conference pages [in Russ.]:
https://fnkcrr.ru/edu/
http://mc.msu.ru/specialist/calendar_of_events/detail/?ELEMENT_ID=7747

Conference Format: Hybrid (in-person and online) [in Russ.]
In-Person Venue:
Institute of Higher and Continuing Education, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25 Petrovka St., Bldg. 2, Moscow, Russia
Online Platform: www.kontur.ru
Online Participant Registration: https://fnkcrr.ru/edu/
Live Q&A and Feedback Tool: «Chat Broadcast»

Participation is free for all attendees, both in-person and online.

For Organizational Inquiries:
Secretariat: +7 (495) 547-13-07, +7 (903) 122-92-80
Email: edu@fnkcrr.ru, a.atlaskirov@fnkcrr.ru
Technical Support for Online Streaming:
Email: talk-help@skbkontur.ru

Conference Media Partners:
- Russian Assembly of Professors
- Bioethics Committee of the Commission for UNESCO Affairs in the Russian Federation
- International Medical University of Integrative and Anti-Aging Biotechnologies

Best regards,
Conference Secretariat

ФЕДЕРАЛЬНОЕ ГОСУДАРСТВЕННОЕ БЮДЖЕТНОЕ НАУЧНОЕ УЧРЕЖДЕНИЕ
"Федеральный научно-клинический центр реаниматологии и реабилитологии"
ФНКЦ РР

Федеральное государственное бюджетное научное учреждение **«Федеральный научно-клинический центр реаниматологии и реабилитологии» (ФНКЦ РР)**

Симуляционный центр ФНКЦ РР

Лаборатория перспективных симуляционных технологий

СИМУЛЯЦИОННЫЕ ОБРАЗОВАТЕЛЬНЫЕ ПРОГРАММЫ:

/ Первая помощь
/ Подготовка инструкторов первой помощи
/ Базовая сердечно-легочная реанимация
/ Расширенная сердечно-легочная реанимация
/ Ультразвуковой мониторинг и навигация в анестезиологии-реаниматологии
/ Трудный дыхательный путь
/ Респираторная поддержка
/ Критические состояния в анестезиологии-реаниматологии
/ Подготовка к первичной специализированной аккредитации
/ Обучение преподавателей симуляционных центров

Все образовательные программы обеспечены баллами НМО
Возможно формирование образовательных циклов по требованию

Тел.: 8(495)650-96-77
www.fnkcrr.ru/edu
edu@fnkcrr.ru