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№ 6

Dear colleagues and partners!

The editorial staff of the peer-reviewed scientific and practical journal «General Reanimatology» appreciate your fruitful collaboration with us in 2025.

Thanks to your professional contribution, the journal «General Reanimatology» enters the coming year with improved performance in major scientometric databases compared to 2024:

• **Scopus,** *Critical Care and Intensive Care Medicine* — Q3; CS=1.9
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• **White List,** *Major (Russian Federation) List of Scientific Publications* — Level 1
• **RSCI,** *IF 5 years* — 1.530
• **Science Index,** *Medicine and Healthcare* — 17th (percentile)
• **VAK** *(Anesthesiology and Resuscitation, 3.1.12; Pathological Physiology, 3.3.3), the Official list of editions recommended for publication of dissertations (PhD, DSci) by the Russian Higher Attestation Commission* — K1

Compared to the previous year, the number of articles downloaded from the journal's website www.reanimatology.com increased by 2.6 times, confirming the interest in the published content and the relevance of the open access model used by the journal.

This year, publications of clinical and experimental studies made up 65% of the content; practical materials — 17.5%, reviews — 15%. About 7% of the published articles were prepared by foreign authors and about 2% papers were submitted by Russian authors jointly with foreign authors. As the author's geography expands, the journal has gained 211 new readers.

This year, the founder of the journal «General Reanimatology», the non-profit organization «Critical Care Medicine Foundation», was admitted to the Association of Scientific Editors and Publishers of Russia. This membership improves the responsibility of the journal's Editorial Board and Editors and provides novel opportunities in developing best editorial and publishing practices and enhancing the journal's development based on leveraging the experience of the expert community.

We congratulate our colleagues and partners on the upcoming 2026!
We wish them good health and a prosperous and successful New Year!

We invite authors and reviewers to fruitful and mutually beneficial collaboration!

Sincerely,
the Editorial Staff
of the journal «General Reanimatology»

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Multimodal LPS-Selective Hemoadsorption and Hemodiafiltration in the Intensive Care of Gram-Negative Sepsis Patients: a Multicenter Observational Study

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Summary

The aim of the study. To evaluate the effectiveness of multimodal selective lipopolysaccharide (LPS) hemoadsorption in combination with renal replacement therapy (RRT) in patients with gram-negative sepsis or septic shock.

Materials and methods. The study included 39 patients. In the main prospective group, patients received extracorporeal therapy in addition to standard of care (ET group, $N=10$). In the retrospective comparison group, patients received only standard therapy (ST group, $N=29$).

Results. In the ET group, the average SOFA score decreased by 3.4 [95% CI: 0.8; 5.2] scores after 72 hours of treatment, while in the ST group, the average SOFA value increased by 1.7 [95% CI: 0; 3.4] scores over the same period, $p_{adj}=0.002$. Hospital mortality was 1/10 (10%) in the ET group and 19/29 (66%) in the ST group, and OR = 0.06 [95% CI: 0.01; 0.4] $P=0.003$. The analysis, including consideration of severity of the condition at a baseline as a potential confounding factor, confirmed the robustness of the results: statistically significant differences in SOFA dynamics and mortality remained.

Conclusion. The use of selective hemoadsorption in combination with renal replacement therapy reduces the severity of organ dysfunction and mortality in patients with sepsis or septic shock.

Keywords: sepsis; septic shock; acute renal failure; endotoxins; hemoadsorption; renal replacement therapy

Conflict of interest. Ismailov E. L. received a speaker fee and travel expenses from PJSC «Efferon». The company did not take part in data analysis and interpretation, as well as in the writing of the manuscript.

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Introduction

Sepsis is not only a problem, but also a serious challenge for international healthcare. Despite all efforts, the mortality rate in septic shock remains high, ranging from 40% to 60% [1]. The lack of significant progress is not only due to antibiotic resistance challenges, but also to the heterogeneity of sepsis, making it too demanding in terms of developing a universal treatment strategy [2]. It is believed that the release of endotoxin and inflammatory mediators into the systemic circulation plays a key role in the pathogenesis of gram-negative sepsis, causing mitochondrial and microcirculatory distress syndrome (MMDS) with associated tissue dysoxia. This is usually accompanied by massive immunocompetent cell death, which exacerbates multiple organ dysfunction syndrome (MODS) [3]. Intestinal paresis and splanchnic hypoperfusion contribute to gut barrier failure with subsequent translocation of bacteria, endotoxins, exotoxins, and microbial metabolites. Increasing concentration of sepsis-associated aromatic microbial metabolites (AMMs) indicates the progression of organ dysfunction and is associated with an increased risk of death [4]. Extracorporeal blood purification in sepsis is a powerful therapeutic tool, its' main goal is to remove pathogen-associated molecular patterns (PAMPs), such as endotoxin, toxic shock syndrome toxin-1 (TSST-1), aromatic microbial metabolites, and damage-associated molecular patterns (DAMPs), such as neutrophil extracellular traps (NETs), cytokines, ammonia, etc., from the systemic circulation.

Standard dialysis membranes and membranes with a high cutoff threshold do not have such properties [5, 6]. Despite the potential of continuous renal replacement therapy (CRRT) to reduce plasma concentrations of certain cytokines, its implementation leads to a loss in serum concentration of most water-soluble antibiotics, amino acids, and some micronutrients, which is very critical for the body [7, 8]. Other modern extracorporeal methods, such as cytokine hemoadsorption, are also being actively studied in critically ill patients. However, to date, the effectiveness of selective cytokine hemoadsorption in sepsis remains highly controversial in the absence of positive results from randomized studies [9, 10].

The most reasonable extracorporeal hemocorrection method in patients with sepsis is selective hemoadsorption of bacterial endotoxins (LPS-selective hemoadsorption) [11–13]. However, the role of lipopolysaccharide hemoadsorption (LPS-adsorption) in the treatment of sepsis has not been definitively established due to insufficient evidence of its positive influence on mortality [14–16].

Recently, a new device for hemoadsorption, Efferon LPS (PAO Efferon, Russia), has been introduced into clinical practice [12]. It is capable of multimodal removal of lipopolysaccharides (due to the LPS-selective ligand immobilized on the surface), excess cytokines, and cytolysis products (due to its internal porosity). The aim of this study was to evaluate the efficacy of multimodal selective LPS hemoadsorption in combination with renal replacement therapy in patients with gram-negative sepsis or septic shock.

Materials and Methods

Study design. The effectiveness of extracorporeal therapy (ET) was evaluated by comparing the prospective (main group, receiving extracorporeal therapy — ET) and retrospective (comparison group receiving standard therapy — ST) cohorts of patients with gram-negative sepsis or septic shock. Patients were recruited from 5 leading multidisciplinary medical institutions in the Republic of Kazakhstan (listed in the «Patients» section). The study was conducted under the supervision of employees of S. D. Asfendiyarov Kazakh National Medical University in the period from 09.2022 to 09.2023. The study was approved by the local Ethics Committee of the Research International Institute of postgraduate education (Almaty, Republic of Kazakhstan), Protocol No. 1 dated April 28, 2022.

Patients. The study included 39 patients with gram-negative sepsis or septic shock, according to the Sepsis-3 criteria [1]. Patients were recruited from the following medical facilities:

1) City Clinical Hospital No. 7, Almaty (ET group, $N=4$; ST group, $N=10$).

2) Aktobe Medical Center, Aktobe (ET group, $N=2$; ST group, $N=7$),

3) City Hospital No. 2, Shymkent (ET group, $N=2$; ST group, $N=6$),

4) City Multidisciplinary Hospital, Taraz (ET group, $N=1$; ST group, $N=4$),

5) Regional Perinatal Center, Kyzylorda (ET group, $N=1$; ST group, $N=2$).

All patients were admitted to intensive care units in a critical condition and received standard intensive care, in accordance with the recommendations of the Surviving Sepsis Campaign 2021 [16].

The main (prospective) group included patients receiving multimodal LPS-selective hemoadsorption (Efferon LPS device, Efferon PAO, Russia) and continuous venovenous hemodiafiltration (ET group, $N=10$) in addition to standard treatment for septic shock. The retrospective comparison group included patients who received only standard therapy (ST group, $N=29$). They were evaluated retrospectively up to 6 months before the first use of Efferon LPS.

Inclusion criteria. Compliance with the criteria for sepsis and septic shock according to the Sepsis-3 definitions at the time of inclusion in the study, followed by identification of pathogen through bacteriological tests; age $\geqslant 18$ years; patient's condition

allowing for the use of Efferon LPS hemoadsorption for at least 6 hours; and informed consent to participate in the study.

Exclusion criteria. Acute blood loss in the last 24 hours, severe granulocytopenia (white blood cell count < 500 cells/mm³) or severe thrombocytopenia (platelet count < 30,000 cells/mm³); HIV infection, terminal renal failure (GFR less than 15 ml/min/1.73 m²); severe congestive heart failure (IV NYHA class, ejection fraction < 35%); acute pulmonary embolism confirmed on CT; acute myocardial infarction within the last 4 weeks; acute cerebrovascular accident; transfusion reaction, anaphylactic reaction, dementia; more than 12 hours after septic shock onset according to Sepsis-3 criteria. Patients without bacteremia (confirmed gram-negative bacteria) were also excluded from the study.

Therapy. All patients received timely intravenous fluids, early treatment with broad-spectrum antibiotics, and vasopressor support as needed. The infusion load for patients in both groups was monitored using dynamic tests (passive leg elevation and pulse pressure variation).

In the ET group, multimodal LPS-selective hemoadsorption with Efferon LPS in combination with continuous venovenous hemodiafiltration (CVVHDF) was initiated within 12 hours from the diagnosis of sepsis or septic shock. Extracorporeal blood purification consisted of 2 consecutive sessions of multimodal LPS-selective hemoadsorption in combination with CVVHDF lasting 6–12 hr, and prescribed effluent fluid rate 30 ml/kg/hr. The second session was performed 24 hours after the first procedure.

A combined extracorporeal circuit with hemoadsorption and CVVHDF was assembled using the PrismaFlex system (Baxter, USA), and the prescribed effluent dose was 30 ml/kg/h. In the extracorporeal circuit, the adsorber was installed after the hemofilter. 2000 ml of saline solution with 5000 IU of unfractionated heparin (UFH) was added to the extracorporeal circuit. Before the procedure, heparin was also administered i/v in a bolus dose of up to 5,000 IU for anticoagulation. UFH was supplied at 500–2,000 IU per hour to maintain the activated partial thromboplastin time (APTT) at twice the normal value. When using Efferon LPS, the blood perfusion rate through the Efferon LPS column was maintained at 150–200 ml/min, and the average duration of perfusion was 6 hours.

No side effects such as intra-procedural hypotension, bleeding, or extracorporeal circuit thrombosis were revealed during the extracorporeal hemodiafiltration (HDF).

A clinical and laboratory examination of the patients was performed before the start of ET and 24 hours after its completion. To monitor the severity of inflammation and endogenous intoxication, the following parameters were assessed: SOFA score, blood, C-reactive protein (CRP), procalcitonin (PCT, measured by quantitative immunochemiluminescence), and the neutrophil-to-lymphocyte ratio (NLR). Blood culture tests were used to detect and identify the causative pathogen. The criteria for initiating RRT in both groups were in compliance with the KDIGO recommendations, 2012 [17].

Statistical analysis. RStudio 2023 and R version 4.2 were used for data analysis and plotting. Data with a normal distribution were presented as *M (SD)*. Data with a log-normal distribution were presented as *Me (Q1; Q3),* and the asymptotic estimation was applied to the log-normal distribution. The *n/N* (%) proportion was presented for categorical data, and for the chi-square test the bootstrapped *p*-values were used (B = 10000). A multi-response permutation procedure technique was used to analyze data with multiple traits (diseases and microbial pathogens). The variables in the groups were compared using Welch's *t*-test, and the log-normal data were logarithmically transformed. The distribution type was determined using the Shapiro–Wilk *W*-statistic. The dynamics of clinical and laboratory indicators over a 72-hour period were analyzed using linear regression with repeated measurements. The data were presented as the mean value and 95% confidence interval, *M* [95% CI]. Age and lactate were considered as covariates. Survival analysis was performed taking into account competing risks (discharge/death), using Fine-Gray regression and the tidycmprsk library. Mortality was also analyzed using logistic regression. Given the observational nature of the study and some baseline differences in patients, as part of the sensitivity analysis, all statistical calculations were repeated with adjustment for covariates. The resulting estimates are presented with the subscript adj. (adjusted).

Results

Clinical parameters before treatment.

The patient selection algorithm is presented in Fig. 1.

No statistically significant differences were found between the groups in terms of age, gender, or anthropometric data (Table 1).

The causes of sepsis or septic shock were infections after abdominal surgery, urological infections, obstetric infections, pulmonary infections, soft tissue infections, bloodstream infections, and infections of the central nervous system (Table 1). Both groups were comparable in terms of severity, although the ET group patients had slightly more severe cardiovascular, respiratory, and renal dysfunction, only the lactate concentration diverged significantly. At the time of inclusion in the study, 9/10 (90%) patients in the ET group and 26/29 (90%) patients in the ST group were diagnosed with septic shock.

Gram-negative bacteremia was documented in 90% of patients in the ET group, compared to 83% in

the ST group (Table 1). These results indirectly indicated the presence of endotoxin in the blood.

The groups were comparable in terms of concomitant diseases (Table 2). The cumulative fluid balance and central venous pressure dynamics in the ICU did not differ between the groups.

Dynamics of clinical parameters after treatment.

A significant improvement in clinical and laboratory parameters was documented in the ET group after the first Efferon LPS therapy session: a rapid decrease in the SOFA score, with an average change of –3.0 [–5.2; –0.8] scores over 72 hours of therapy, while the ST group demonstrated worsening of multiple organ failure — M=+1.3 [–0.6; +3.3], and the differences between the groups were statistically significant, $P=0.005$ (Table 3). Simultaneously, significant modifications in the doses of administered vasopressors were documented using VIS2020 scale [18], in the ET group it was –11 [–17; —5.5] in 72 hours, and in the ST group +11 [–4; +26], $P=0.008$. The decrease in lactate was more marked in the ET group vs the ST group: –3.2 [–4.6; –1.8] and –0.7 [–1.6; +0.2], respectively, $P=0.004$. Sensitivity analysis in multivariate linear regression taking into account covariates (age and lactate concentration) showed similar results (Table 3).

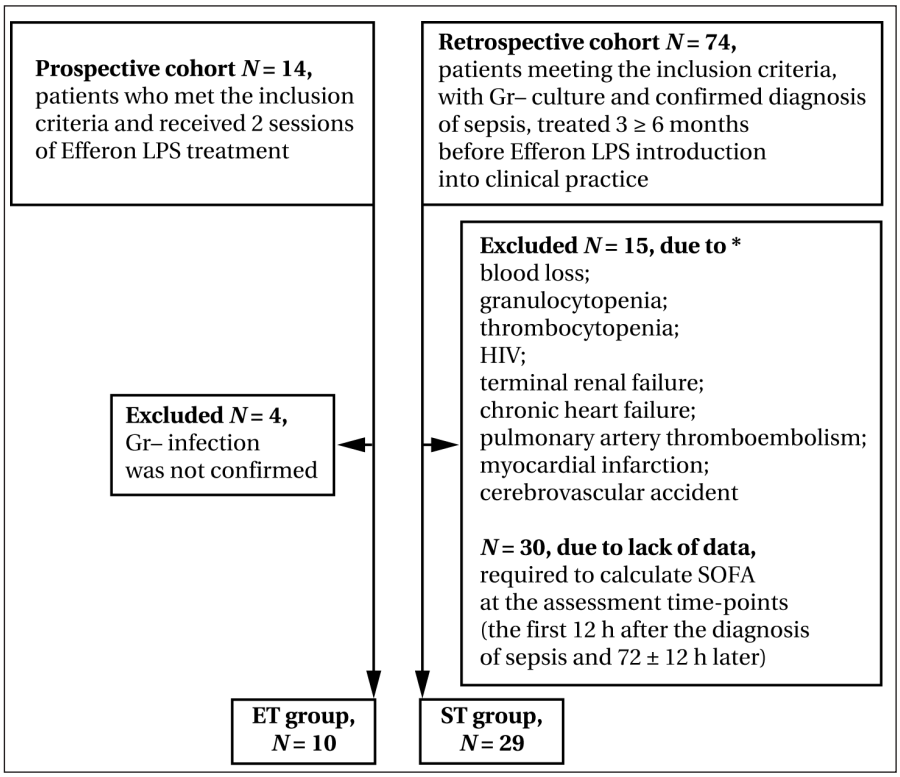

Fig. 1. Patient selection algorithm.
Note. * — a detailed description is provided in the «Materials and Methods» section.

Positive changes in the clinical picture included regression of the SOFA score, a decrease in body temperature to subfebrile values and the severity of pain syndrome, the appearance of peristaltic sounds, improved respiratory function and oxygenation with an increase in PaO_2/FiO_2, recovery of hemodynamic stability with reduced administration of vasopressors, and rebound diuresis, allowing to discontinue RRT after 3 days.

The dynamics of C-reactive protein and procalcitonin levels indirectly confirmed a decrease in endogenous intoxication (Table 3). Complete stabilization of laboratory parameters was documented by the 7th day of treatment.

In the ET group, 9 out of 10 patients had septic shock upon admission, one more patient developed shock on the third day after inclusion in the study. In the ST group, 90% (26/29) of patients had septic shock upon admission to the ICU, and the remaining 3 patients developed it later. Septic shock was resolved in 89% (8/9) of patients in the ET group and in 38% (10/26) of patients in the ST group, OR=13 [95% CI: 1.9; 257] $P=0.018$, $O_{Radj.}=30$ [95% CI: 3.4; 806] $P=0.010$, (Fig. 2, *a*). The incidence curves for the resolution of septic shock, acute kidney injury, transfer from the ICU, and ICU mortality differed significantly between the groups, both with and without adjustment for covariates (Fig. 2). The overall mortality rate was 20/29 (69%) in the ST group and 1/10 (10%) in the ET group, OR=0.06 [95% CI: 0.01; 0.4] $P=0.003$, $OR_{adj.}=0.03$ [95% CI: 0.01; 0.2] $P=0.005$. The duration of ICU stay did not differ statistically between the groups.

Discussion

The mortality rate for septic shock remains significant worldwide. According to a review, the 30-day mortality rate for patients with septic shock in Europe, North America, and Australia is 34.7% on average [19]. According to a meta-analysis by Chinese authors, the overall 28–30-day mortality rate for sepsis and septic shock was 37.3% [20].

According to our data, this is the first study in Kazakhstan to investigate the effectiveness of combined multimodal LPS-selective hemoadsorption and CVVHDF in patients with sepsis or septic shock. The 28-day mortality rate in the ST group was 66%, compared to 10% in the ET group.

Table 1. Patient characteristics at the time of inclusion in the study.

Parameters	Values in the groups, *M (SD) / n (%) / Me (IQR)*		*p*
	ET (*n* = 10)	ST (*n* = 29)	
Age, years	52 (14)	54 (18)	0.663
Body weight, kg	76 (14)	72 (14)	0.470
SOFA, scores	8 (3)	7 (4)	0.646
MAP, mmHg	74 (14)	78 (22)	0.504
Septic shock, *n/N* (%)	9/10 (90%)	26/29 (90%)	1
VIS 2020*, units	18 (12; 22)	10 (0.1; 17)	0.144
ARF, n/N (%)	8/10 (80%)	14/29 (48%)	0.14
MV, n/N (%)	4/10 (40%)	8/29 (28%)	0.702
PaO_2/FiO_2,	245 (89)	218 (71)	0.563
AKI, n/N (%)	10/10 (100%)	22/29 (76%)	0.234
Need for RRT, *n/N* (%)	10/10 (100%)	20/29 (69%)	0.116
Diuresis, ml/day	253 (34; 1847)	249 (41; 1493)	0.989
Creatinine, μmol/L	298 (141)	219 (184)	0.177
WBC, $\times 10^9$/L	19.9 (9.1)	17.9 (9.7)	0.563
Neutrophils, $\times 10^9$/L	17 (9)	15 (9)	0.470
Lymphocytes, $\times 10^9$/L	1.09 (0.7; 1.56)	1.06 (0.53;1.79)	0.926
NLR,	15.6 (8.4; 28.3)	13.9 (6.5; 28.7)	0.962
CRP, mg/L	258 (145)	178 (111)	0.140
Procalcitonin, ng/ml	14.1 (4; 44.2)	8.8 (2.4; 26.8)	0.483
Total bilirubin, μmol/L	39 (16; 94)	23 (12; 45)	0.265
Lactate, mmol/L	5.2 (2.1)	3.5 (2.2)	0.047
Platelets, $\times 10^9$/L	192 (108)	217 (124)	0.556
INR, units	1.37 (1.21; 1.54)	1.59 (1.26; 1.97)	0.077
Fibrinogen, g/L	5.9 (2)	4.7 (2.7)	0.164
Bacteremia, *n/N* (%)	9 (90%)	24 (83%)	1
Primary infectious site, *n/N* (%)			0.289
Acute pyelonephritis	2 (20%)	5 (17%)	
Acute pancreatitis	5 (50%)	3 (10%)	
Pneumonia	1 (10%)	9 (31%)	
Acute cholecystitis	0 (0%)	2 (7%)	
Acute intestinal obstruction	0 (0%)	2 (7%)	
Cellulitis	1 (10%)	3 (10%)	
Other	1 (10%)	5 (17%)	
Etiology of sepsis, *n/N* (%)			0.832
Gram-negative bacteria	10 (100%)	29 (100%)	
Klebsiella spp.	5 (50%)	16 (55%)	
Escherichia coli	3 (30%)	7 (24%)	
Acinetobacter spp.	1 (10%)	9 (31%)	
Pseudomonas aeruginosa	2 (20%)	6 (21%)	
Enterobacter spp.	1 (10%)	3 (10%)	
Proteus ssp.	0 (0%)	2 (7%)	
Gram-poisitive bacteria	2 (20%)	6 (21%)	
Staphylococcus aureus	1 (10%)	4 (14%)	
Streptococcus spp.	1 (10%)	1 (3%)	
Enterococcus spp.	1 (10%)	2 (7%)	

Note. Here and Table 3: *VIS2020 — Vasoactive Inotropic score [18]. *M (SD)* — mean value and standard deviation; *Me (Q1; Q3)* — median, 1 and 2 quantiles; SOFA — the Sequential Organ Failure Assessment scale; MAP — mean arterial pressure; NLR — neutrophil-to-lymphocyte ratio; INR — international normalized ratio; RRT — renal replacement therapy; ET — extracorporeal therapy; MV — mechanical ventilation; AKI — acute kidney injury.

Most of the patients included in the study had bacteremia, i. e. 83% in the ST group and 90% in the ET group. Microbiological examination of biological samples is a prerequisite for effective antibiotic therapy. Gram-negative pathogen was isolated and bacteriologically confirmed in 100% of patients in both groups. Gram-positive bacteria were found in 21% (ET group) and 20% (ST group), indicating a mixed type infection involving both Gram-positive and Gram-negative bacteria (Table 1). In the EUPHRATES study, isolated Gram-negative flora was found in 23.9% of cases in the selective LPS hemoadsorption group, compared to 13.3% in the comparison group [22].

Bacteremia in sepsis can worsen the prognosis for patients. For example, a 2023 review of the significance of bacteremia in 258 septic patients showed that bacteremia in patients with sepsis was associated with higher mortality rates and longer intensive care unit stays compared to patients without bacteremia, although this difference did not reach statistical significance [21].

The LASSO RCT (2021–2022 yrs, Russia) established an interesting fact of positive correlation between procalcitonin concentration, severity of endotoxemia, and SOFA score ($P < 0.05$) [12]. The LASSO data suggest that the main group patients had higher concentrations of endotoxin compared to the control group, based on documented in these patients higher levels of procalcitonin, CRP, and lactate at the time of inclusion [23].

The Efferon LPS machine is a multimodal device [24]. Due to immobilized on the surface of sorbent granules' LPS-binding ligand and granules' porous structure, the sorbent is capable of simultaneously removing both bacterial endotoxins and endogenous inflammatory mediators (cytokines and cytolysis products) [12].

A recent meta-analysis of the effects of extracorporeal blood purification methods on mortality, need for RRT, mechanical ventilation, and duration of hospital stay demonstrated that the use of continuous venovenous hemofiltration (CVVH) and concomitant use of CVVH and cytokine hemoadsorption led to reduced ICU stay [25]. In our study, the effect of eliminating LPS and DAMP molecules using Efferon LPS was potentiated by a 6-hour CVVHDF procedure.

In RCTs conducted before 2020 hemoadsorption was usually initiated in patients with average SOFA score of $\geqslant 10$ [26, 27]. An increase in survival and organ-support free days after the use of selective Polymyxin B hemoadsorption was demonstrated in 2191 patients out of 44177 analyzed sepsis cases from the National database (2018–2020 yrs) in a review by Japanese authors. [28]. The greatest advantages were documented in patients with baseline SOFA score of 7–12. The need for timely selective hemoadsorption using polymyxin B hemoperfusion was confirmed in a review describing studies in more than 2,000 patients [28]. According to the review, statistically significant positive results of extracorporeal treatment were obtained only when the SOFA score values of patients was within the range of 7–12 scores. In our study, the

Table 2. Distribution of chronic diseases.

Nosological diagnosis	Distribution in the groups	
	ET, N=10	ST, N=29
Diabetes mellitus	2 (20%)	9 (31%)
Arterial hypertension	3 (30%)	11 (38%)
Chronic kidney disease	4 (40%)	9 (31%)
Nephrolithiasis	1 (10%)	1 (3%)
Congestive heart failure/coronary artery disease	3 (30%)	5 (17%)
Chronic cholecystitis/gallstone disease	1 (10%)	1 (3%)
Anemia	3 (30%)	4 (14%)
Chronic gastric ulcer/gastritis	1 (10%)	2 (7%)
Chronic obstructive pulmonary disease/respiratory failure	2 (20%)	6 (21%)
Chronic cerebrovascular disease/strioke/discirculatory encephalopathy	1 (10%)	3 (10%)
Liver cirrhosis/hepatitis	1 (10%)	2 (7%)
Obesity	2 (20%)	2 (7%)
Other	2 (20%)	4 (14%)
p	0.439	

Table 3. Dynamics of parameters over 72 hours of treatment: ET group vs ST.

Parameters	Groups	Interval 0–72 hr			
		One-dimensional model M [95% CI]	p	Multi-dimensional model $M_{adj.}$ [95% CI]	p
SOFA	ET	–3.0 [–5.2; –0.8]	0.005	–3.4 [–5.2; –0.8]	0.002
	ST	+1.3 [–0.6; +3.3]		+1.7 [–0.0; +3.4]	
MAP	ET	+10.2 [+2; +18.4]	0.244	+7.8 [–2.1; 17.6]	0.476
	ST	+1.1 [–12.1; +14.3]		+3.4 [–3.4; 10.3]	
VIS2020	ET	–11.4 [–17.3; –5.5]	0.008	–13.7 [–30.6; 3.1]	0.005
	ST	+10.9 [–3.9; +25.6]		+19.2 [5.8; 32.7]	
PaO_2/FiO_2	ET	+108 [–8; +225]	0.081	+106.2 [–6.1; 218.5]	0.119
	ST	–5.5 [–61.7; +50.7]		–1 [–66.2; 64.2]	
Lactate	ET	–3.2 [–4.6; –1.8]	0.004	–2.6 [–3.6; –1.5]	0.02
	ST	–0.7 [–1.6; +0.2]		–1 [–1.7; –0.3]	
Total bilirubin	ET	–21.3 [–36; –6.7]	0.012	–16.8 [–31; –2.7]	0.067
	ST	+2.5 [–8.1; +13.2]		–0.4 [–10.1; 9.3]	
Diuresis	ET	+870 [+122; +1618]	0.352	+897 [+141; +1653]	0.245
	ST	+443 [–98; +984]		+325 [–265; +914]	
Creatinine	ET	–112.7 [–219.5; –6]	0.095	–93.8 [–174.2; –13.4]	0.084
	ST	–8.3 [–70.5; +53.9]		–6.8 [–61.9; +48.4]	
WBCs	ET	–7 [–12.6; –1.4]	0.403	–5.7 [–9.7; –1.8]	0.755
	ST	–4.3 [–7.6; –1]		–5 [–7.7; –2.3]	
NLR	ET	–5.9 [–18.1; +6.2]	0.625	–6.7 [–11.5; –1.9]	0.198
	ST	–9.3 [–16.2; –2.4]		–10.5 [–13.8; –7.2]	
Platelets	ET	–86 [–138; –33]	0.339	–89 [–130; –48]	0.147
	ST	–54 [–94; –15]		–52 [–79; –25]	
CRP	ET	–81.8 [–153.5; –10.2]	0.096	–61.4 [–107; –15.8]	0.169
	ST	–16.4 [–46.9; +14.1]		–21.8 [–53.9; +10.3]	
PCT	ET	–22.3 [–41.1; –3.6]	0.091	–15.2 [–24.3; –6.1]	0.320
	ST	–3.3 [–15.3; +8.8]		–9.3 [–16.3; –2.3]	
Lymphocytes	ET	–0.2 [–0.9; +0.5]	0.816	–0.2 [–0.9; 0.5]	0.320
	ST	–0.1 [–0.6; +0.5]		–0.1 [–0.6; +0.4]	
Neutrophils	ET	–6.4 [–12; –0.8]	0.471	–5.1 [–8.4; –1.7]	0.954
	ST	–4.2 [–6.9; –1.4]		–4.9 [–7.2; –2.6]	

Note. PCT — procalcitonin; MAP — mean arterial pressure.

average SOFA score in the ET group was 7.9, falling within the same SOFA range, when the hemoadsorption yields maximum efficiency.

The study had a number of limitations. Firstly, the ST comparison group was selected retrospectively, based on archived medical files therefore, cases were not initially adapted for the study, which made it impossible to compare a number of parameters. For example, the magnitude of multimodal LPS-selective hemoadsorption and CVVHDF positive effect on systemic blood pressure, on severity of vascular insufficiency, and cardiac index, due to the lack of invasive hemodynamic monitoring in ST group patients. Circulating endotoxin activity and concentrations were also not evaluated. Secondly, the statistical power was limited by the small sample size. Thirdly, the ST group patients did not receive CVVHDF, making it impossible to separate the effects of endotoxin sorption and CVVHDF. Additionally, the regression analysis used to adjust for the heterogeneity of patient baseline characteristics between groups has its own limitations.

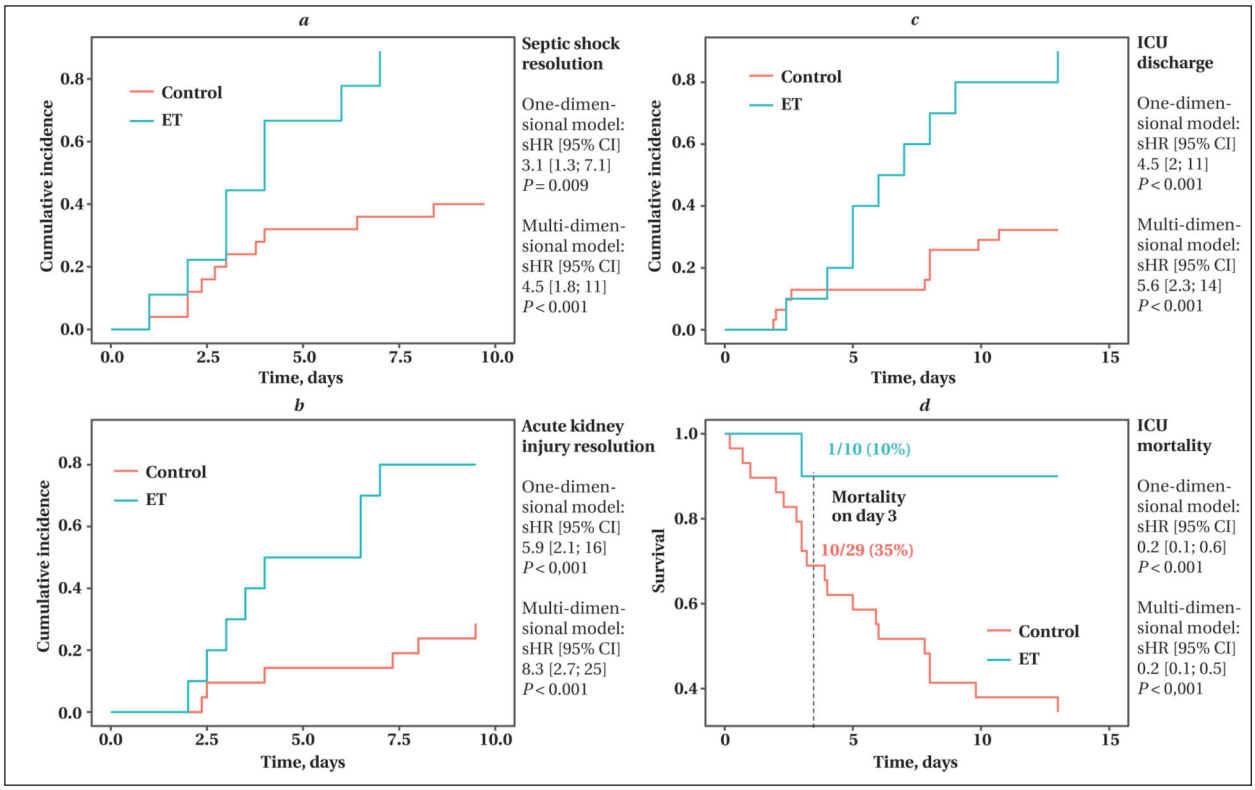

Fig. 2. Incident curves of septic shock resolution (*a*), acute kidney injury resolution (*b*), ICU discharge (*c*), and ICU mortality (*d*).

Conclusion

The concomitant use of selective hemoadsorption and hemodiafiltration in patients with sepsis or septic shock reduces the severity of organ dysfunction and mortality.

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Effect of Renal Filtration Function on Polymyxin B Pharmacokinetics in Patients with Sepsis

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Summary

The aim of this study was to investigate pharmacokinetics of polymyxin B (PMB) in patients with sepsis and preserved or impaired renal function.

Methods. A two-center, prospective, randomized study enrolled patients with sepsis. Blood samples for analysis were collected in a steady state (SS) to assess the pharmacokinetics of PMB. PMB concentration in the patients' serum was measured using a direct competitive ELISA to verify the achievement of target area under the concentration-time curve (AUC_{24}) value of 50–100 mg×h/L.

Results. The study included 34 patients with sepsis receiving PMB therapy who were distributed into two groups based on their glomerular filtration rate (GFR): patients with impaired renal function (GFR < 80 mL/min, $N = 17$), and with preserved renal function (GFR ⩾ 80 mL/min, $N = 17$). The mean AUC_{24} value in 34 patients was 64.02 ± 11.64 mg×h/L, the median volume of distribution was 31.53 (23.79–43.72), and the median clearance was 3.72 (2.73–4.85). In patients with preserved renal function, the median AUC_{24} was 48.38 mg×h/L, and the SS concentration was 2.02 mg/mL. In patients with impaired renal function, the AUC_{24} was 71.78 mg×h/L, and the SS concentration was 2.99 mg/mL. The clearance of PMB differed considerably depending on GFR: the median clearance in patients with GFR < 80 mL/min was 3.28 L/h versus 4.97 L/h in patients with GFR > 80 mL/min ($P = 0.012$). In 14 of 17 patients with reduced renal function, the AUC_{24} values fell in the range of 50–100 mg×h/L. In the group with preserved and increased renal function, only 7 of 17 patients reached the target range, and in 53% of patients (9 of 17), the exposure to PMB was below the therapeutic level (AUC_{24} < 50 mg×h/L).

Conclusion. Renal function affects the clearance of PMB and, consequently, the achievement of the target therapeutic range. Standard dosing regimens do not provide achieving the target concentrations of antibiotic in all patients, as some patients with preserved renal function have insufficient PMB exposure, while patients with impaired renal function have enhanced exposure, increasing the risk of drug failure or toxicity. To ensure the effectiveness and safety of treatment, regular therapeutic PMB concentrations monitoring and individual dose adjustments are necessary, especially in patients with altered renal function.

Keywords: renal filtration function; polymyxin B; polymyxin B pharmacokinetics; sepsis; antibacterial therapy

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

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Introduction

Polymyxins, colistin and polymyxin B (PMB) are polypeptide antibiotics with a unique mechanism of action that involves disrupting the integrity of the outer membrane of gram-negative bacteria, which enhances the activity of other classes of antibacterial drugs. This class of antibiotics was commonly used in clinical practice for treatment of infections caused by multydrug resistant (MDR) *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Enterobacteriaceae* strains since the 80s of the last century. Most invasive gram-negative infections currently remain sensitive to PMB, which explains its effectiveness [1]. The «International consensus

guidelines for the optimal use of the polymyxins» [2] recommend to observe the following pharmacokinetic (PK) parameters during treatment of infections caused by MDR gram-negative bacteria: AUC_{24h}=50–100 mg×h/L (area under the serum PMB concentration curve at steady state (SS) over 24 hours), which corresponds to C_{ss}=2–4 mg/l (average steady-state concentration). The recommended AUC_{24h} range is a predictor of maximum clinical efficacy with minimal likelihood of toxic events. According to prevailing consensus PMB exposure exceeding 100 mg×h/L increases the risk of adverse reactions, mainly acute renal injury [3]. However, a study by Z. Yu, et al., involving 312 patients, showed that there was no correlation between an increase in AUC_{24h} > 100 mg×h/L and the incidence of acute kidney injury, and that mortality increased at AUC_{24h} < 50 mg×h/L [4]. However, the criteria for achieving PMB clinical efficacy have not yet been established [5].

Critical conditions, such as sepsis, are accompanied by serious pathophysiological alterations that lead to significant changes in pharmacokinetics. The presence of chronic concomitant pathology can exacerbate pathophysiological changes, significantly affecting the pharmacokinetics of antibacterial agents.

Excess body weight, endothelial dysfunction, massive capillary leakage syndrome, and extensive infusion therapy can modify the volume of distribution (V_d). Changes in drug clearance (CL) occur due to increased renal clearance, impaired renal function, and the use of extracorporeal treatments and extracorporeal life support (ECLS). Additionally, changes in the blood plasma protein composition, including hypoalbuminemia, can increase V_d due to increased circulating free drug fraction leading indirectly to increased clearance [6].

Demographic data (patient weight and age), biochemical parameters (albumin and creatinine concentrations), and SOFA scores as organ dysfunction measure in sepsis are considered as covariates in the analysis of PK parameters [7]. Most often, PMB dosing is based on the patient's body weight. In a review of 10 PMB PK studies, plasma albumin levels were not considered as a significant covariate [8]. Kidney function statistically significantly affects PK parameters [9]. For example, at a glomerular filtration rate (GFR) of >80 ml/min, adequate plasma concentration of colistin (structurally similar to polymyxin) was attained in less than 40% of cases even at the maximum recommended daily dose because of active renal excretion [10].

Dose-dependent nephrotoxicity is a major PMB –associated side effect [11, 12]. It has been established that PMB accumulates in the renal cortex [13]. Although PMB is mainly eliminated by non-renal routes (mostly via biliary exretion) [14], the excretion of unchanged antibiotic in the urine can vary significantly among individuals, ranging from 0.98% to 17.4% [15].

The choice of the optimal PMB dosing regimen is still a matter of debate, even for susceptible pathogens with MIC (minimum inhibitory concentration) ⩽2 mg/L. The narrow therapeutic window requires a balance between therapeutic efficacy and the risk of complications. Previously, a study of PMB PK in patients with ECMO support showed that the recommended standard dosing regimens of 2.5–3.0 mg/kg/day were adequate, and no increase in dosage was required [16]. PMB-associated nephrotoxicity requires monitoring of patient's kidney function, but according to some researchers, renal dysfunction is not an indication for dose adjustment [17, 18]. Other researchers conclude that it is necessary to reduce doses in renal failure, but in severe infections caused by microorganisms with PMB MICs of ⩾2 mg/L, they allow the use of a high daily dose of 200 mg/day [19], as reduction of the dose increases the rate of fatal outcomes. All studies show significant interindividual variability in PMB PK. A comparative study of PMB PK in patients with preserved and impaired renal function is noteworthy, demonstrating that renal function contributes to PMB clearance [20]. All of the above indicates the need to implement the principles of personalized medicine into the prevention, diagnosis, and treatment of patients in critical conditions [21].

In recent years, the number of PMB PK studies has increased significantly. In a 2024 review analyzing 22 studies conducted since 2008, 14 reports from China were included [7]. The main cohort consisted of patients with a normal weight (for example, in one of the largest studies, the average weight of patients (N=486) was 70 kg), with a recommended PMB dose of 1.5–3.0 mg/kg/day [3]. A greater weight (>80 kg) was considered obesity, and published data on such patients are limited [22].

In this study, we investigated the PK of PMB and analyzed the relationship between PK parameters and renal function in patients with sepsis in Moscow hospitals, who were predominantly elderly (>60 years) and overweight (BMI ⩾28).

The aim of this study was to investigate pharmacokinetics of polymyxin B (PMB) in patients with sepsis and preserved/impaired renal function.

Materials and Methods

Study design and patients. A two-center, prospective, randomized study was conducted at MEDSI Clinical Hospital No. 1 in 2021–2022 and at the National Medical Research Center, Medical and Rehabilitation Center of the Russian Ministry of Health in 2025. The study inclusion criteria for patients were as follows: diagnosis of sepsis caused by MDR gram-negative microorganisms according

to the Sepsis-III criteria, PMB therapy lasting at least 48 hours, and age over 18 years. The exclusion criteria for the study were pregnancy and ECLS use (renal replacement therapy, extracorporeal membrane oxygenation). The patient recruitment scheme for the study is shown in Fig. 1.

The clinical study of PMB PK in intensive care patients was approved by the Independent Ethics Committees of MEDSI group of companies (commission meeting protocol No. 29 dated April 15, 2021) and the National medical research center for intensive care of the Ministry of Health of the Russian Federation (commission meeting protocol No. 061 dated January 22, 2025). Voluntary informed consent to participate in the study was obtained from patients or their legal representatives. On the first day, all patients received a PMB loading dose at a rate of 2.5–3.0 mg/kg intravenously once (PMB therapy is recommended to start with a loading dose of 2.0–2.5 mg/kg) [2].

A population pharmacokinetic study in critically ill patients showed that after the first dose of the regimen of 1.25 mg/kg every 12 hours, PMB plasma concentration achieved ~56–70% of the SS concentration values [15]. It was calculated using Monte Carlo simulation, that at a loading dose of 2.0 mg/kg, PMB concentration would be 76–94% of the values observed at steady state [15]. Further PMB therapy was administered twice daily at a dose of 75–150 mg (2.5–3.0 mg/kg/day). The drug was dissolved in 5% glucose and administered intravenously over one hour using a syringe dispenser. The PMB MIC were obtained based on isolates susceptibility testing using the VITEK® 2 automatic analyzer (bioMérieux, France). GFR was estimated using the Cockcroft-Gault equation [23].

Blood sampling. To assess PK PMB at steady state, blood samples were collected before drug administration and then 1.1, 1.5, 2, 3, 5, 9, and 12 hours after the start of the 1-hour infusion. Blood samples were centrifuged at 1600×g for 10 min, the separated serum was immediately frozen and stored at –20°C for 10–90 days prior to analysis.

Solid-phase competitive enzyme-linked immunosorbent assay (ELISA). PMB concentration in serum samples was evaluated using a direct competitive enzyme — linked immunosorbent assay (ELISA) according to previously described method [16, 24]. Sample preparation included preliminary deproteinization of serum samples using 5% trichloroacetic acid. After centrifugation, the supernatant was diluted 100-fold with an assay buffer consisting of 0.15 M phosphate — buffered saline (pH 7.2) containing 0.05% Tween 20. The results of the subsequent immunoassay were available after 1.5–2 hours.

Pharmacokinetic and statistical analysis. Based on the results obtained from measuring PMB concentration in patients' serum, the PK parameters were calculated with Lixoft SAS 2024 software (France) using a non-compartmental method.

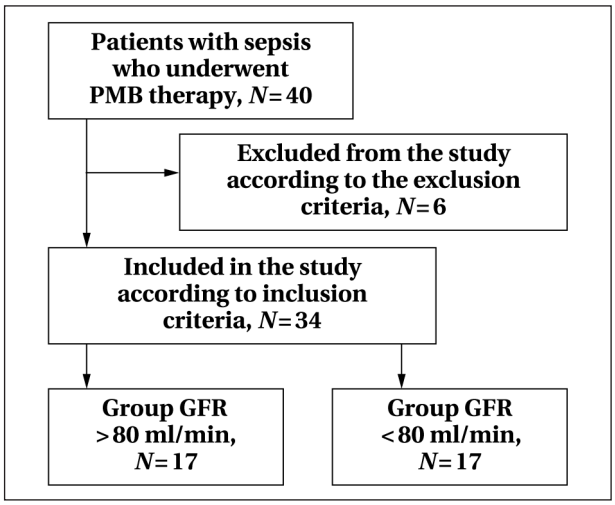

Fig. 1. Patient recruitment scheme for the study.

Primary data analysis and visualization were performed using Microsoft Office Excel 2019 and GraphPad Prism 9 software. Qualitative variables were described statistically as absolute and relative data. The normality of data distribution was assessed using the Shapiro–Wilk test. For quantitative variables, the mean *(M)* and standard deviation *(SD)* were calculated, as well as the median *(Me)* and interquartile range (IQR). Differences between groups with normal distribution of quantitative data were analyzed using Student's *t*-test and Mann–Whitney test for quantitative data that did not follow a normal distribution. Welch's *T*-test was used to determine the statistical difference between the means of two groups with different dispertion. The relationship between two quantitative parameters was assessed using Spearman's correlation. A level of $P < 0.05$ was accepted as the criterion for statistical significance for intergroup comparisons. The data were statistically processed using IBM SPSS Statistics v. 28.0 software.

Study endpoints. The primary endpoint of the study was the frequency of achieving the target AUC_{24} 50–100 mg×h/L at steady state in each group of patients. The secondary endpoints were: PMB distribution volume and clearance, albumin concentration, and median patient weight.

Results

The study included 34 patients with sepsis receiving PMB therapy, who were divided into two groups ($N=17$) according to their GFR. The cutoff value was set at GFR = 80 ml/min [10, 20], with a median GFR for all patients ($N=34$) of 80.7 ml/min (46.5; 125.4). The study included adults (aged 30 up to 89 years old), men and women. Most patients in both groups were men (12/17 and 10/17). The median age in the group of patients with reduced

Table 1. Demographic and clinical characteristics of patients.

Parameters	Glomerular filtration rate, ml/min		*P*
	>80, *N*=17	<80, *N*=17	
	Values		
Gender			
Males, *N* (%)	12 (71)	10 (59)	0.632*
Females, *N* (%)	5 (29)	7 (41)	
Age, years	64 (57; 68)	70 (64; 76)	0.039
Weght, kg	100 (80; 110)	80 (70; 100)	0.022
BMI	33.9 (27.7; 34.0)	28.0 (24.0; 31.1)	0.015
Isolated bacteria			
Klebsiella pneumoniae, N	3	8	
Acinetobacter baumannii, N	7	4	
Pseudomonas aeruginosa, N	1	2	
Mixed flora	6	3	
SOFA	7 (6; 8)	8 (5; 10)	0.715
Total protein, g/l	52.5 (46.8; 59.6)	53.0 (49.0; 60.6)	0.710
Albumin, g/l	26.6 (23.8; 30.0)	26.8 (24.4; 28.2)	0.559
Bilirubin, μmol/L	12.3 (9.6; 32.3)	13.7 (10.5; 28.7)	0.967
Creatinine, μmol/L	72.0 (53.0; 89.0)	175.0 (103.5; 272.5)	<0.001
GFR, ml/min	123.0 (89.2; 159.6)	42.3 (24.3; 64.4)	<0.001

Note. Data are presented as absolute values for qualitative variables — *N* (%), and as *Me* (IQR) *f*or quantitative variables, where *Me* is the median and IQR is the interquartile range; * — *Z*-test of proportions.

GFR was 70 years (range 56– 89 years) with a median body weight of 80 kg (range 64–100 kg). The median age and weight in the group with GFR >80 ml/min were 64 years (range 35–85 years) and 100 kg (range 75–140 kg). Sepsis was mainly caused by carbapenem-resistant strains of *K. pneumonia* (*N*=11), *A. baumannii* (*N*=11), or *P. aeruginosa* (*N*=3), and by multiple pathogens with MIC ⩽ 1 μg/mL in 9 patients. Secondary bacterial pneumonia was diagnosed in 71.9% of cases, bloodstream infection — in 7 cases, and abdominal infection — in 2 patients. Based on the SOFA score, these two groups of patients did not differ in terms of organ dysfunction, total protein, albumin, or bilirubin levels. Patients in both groups differed significantly in terms of creatinine concemtrations and GFR ($P<0.001$). Demographic and clinical data are shown in Table 1.

The patients' (*N*=257) steady-state serum antibiotic concentrations are presented in Fig. 2.

The mean AUC_{24} for 34 patients at steady state was 64.02 ± 11.64 mg×h/L, the median distribution volume was 31.53 (23.79; 43.72), and the median clearance was 3.72 (2.73; 4.85). Preliminary analysis of the data allowed to establish relationships and evaluate the effect of various covariates (age, body weight, SOFA, creatinine clearance, bilirubin, total protein, and albumin levels) on main PK parameters (AUC_{24}, V_d, and CL). Of all the data analyzed, a relationship was found for some pairs. An inverse correlation was established between the volume of distribution and albumin concentration (r_s=–0.355, P=0.039), as well as a significant direct correlation between GFR and clearance (r_s=0.427, P=0.012). The dose-normalized AUC depended on direct correlation with SOFA (r_s=0.453, P=0.007). The relationship between these variables is shown in Fig. 3

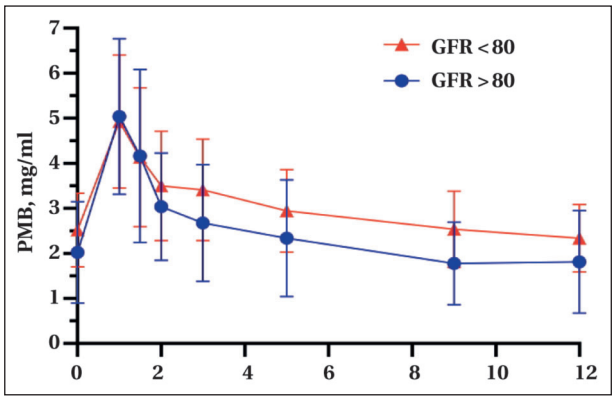

Fig. 2. PMB SS pharmacokinetic profile in patients with sepsis (*N*=34).
Note. Concentration is presented as the mean value ± standard deviation (*N*=17).

Since the albumin concentration and SOFA severity score in patients in both groups were comparable, and the differences in GFR were statistically significant ($P<0.001$), the analysis of PK data in the groups was continued in more detail. PK parameters were calculated based on individual plasma PMB concentrations at different time-points (Table 2).

In patients with preserved renal function, the median AUC_{24} value was 48.38 mg×h/L, and the SS concentration was 2.02 mg/mL. In the group with reduced renal function, the median AUC_{24} value reached 71.50 mg×h/L, and the SS concentration was 2.99 mg/mL. After normalizing the AUC_{24} values by dose, the level of differences between the groups increased statistically (from P=0.027 to P=0.01). At the same time, the PMB C_{max} in patients from both groups was similar — 5.1 mg/mL (P=.931). The PMB volume of distribution was slightly lower in

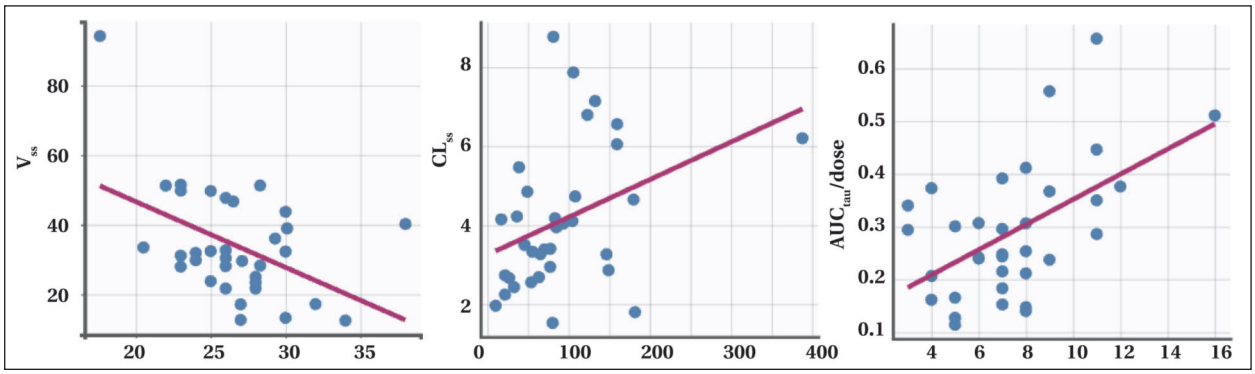

Fig. 3. PMB SS pharmacokinetic profile in patients with sepsis (*N* = 34).
Note. Concentration is presented as the mean value ± standard deviation (*N* = 17).

Table 2. PMB SS PK parameters in patients with different glomerular filtration rates.

Parameters	Glomerular filtration rate, ml/min		*P*
	>80, *N* = 17	<80, *N* = 17	
AUC_{24}, mg×h/L	48.38 (39.42; 65.90)	71.50 (59.60; 79.44)	0.027
AUC_{24}/dose, mg×h/L	0.25 ± 0.15	0.33 ± 0.09	0.010
C_{max}, mg/L	5.11 ± 1.96 (2.24–9.67)	5.09 ± 1.42 (2.35–8.34)	0.931
C_{ss}, mg/L	2.02 (1.70; 2.66)	2.99 (2.48; 3.33)	0.027
CL, l/h	4.97 ± 2.07 (1.52–8.77)	3.28 ± 0.95 (1.96–5.46)	0.012
V_d, L	32.71 (29.22; 48.22)	28.29 (21.71; 34.12)	0.114
T_{max}, h	1.17 ± 0.16	1.19 ± 0.18	0.694

Note. Values are presented as mean ± standard deviation (range) and *Me* (IQR), where *Me* is the median and IQR is the interquartile range.

the group with reduced renal function (28.29 L vs. 32.71 L), but the differences between the groups were statistically insignificant ($P = 0.114$). Clearance values were normally distributed across groups and differed statistically significantly. In the group of patients with GFR < 80 ml/min, the median clearance value was 3.28 L/h, compared to 4.97 L/h in the group of patients with GFR > 80 ml/min ($P = 0.012$). The parameters of total PMB exposure (AUC_{24}) in individual patients showed significant differences (Fig. 4).

In 14 of 17 patients with reduced renal function, AUC_{24} values were in the range of 50–100 mg×h/L. Among patients with normal renal function and hyperfiltration, only 7/17 attained the target range, while in 53% of patients (9/17) PMB exposure did not reach therapeutic efficacy levels (AUC_{24} < 50 mg×h/L).

Discussion

PMB exposure was assessed depending on GFR and renal function. PK was described using a non-compartmental model, which allowed the obtained data to be evaluated.

The PMB pharmacokinetics was described in 34 patients with sepsis, divided into 2 groups depending on renal function (GFR > 80 and < 80 ml/min). Despite differences between the groups in terms of median body weight and age of patients ($P < 0.05$), most of them were overweight (median weight 90 kg, BMI ⩾ 28) and elderly (over 60 years old). Previously, patients with such demographic data had not been described. In a similar study in 2021, patients with increased body weight (> 90 kg) had a mean age of 52 years [22]. In another study, the mean body weight of 23 elderly people was 70 kg [25], and the mean age was 73 years.

The mean AUC_{24} for all patients at steady state was 64.02 ± 11.64 mg×h/L, which is within the target range (50–100 mg×h/L). However, in some patients (2/34, 5.9%), PMB exposure exceeded the toxicity threshold (> 100 mg×h/L), and in 11/34 (32.4%) patients, it was insufficient (< 50 mg×h/L), which increased the risk of treatment failure. The median values of distribution volume and clearance in the respective groups (31.53 L and 3.72 L/h), taking into account the heterogeneity of patients, were comparable to those described earlier [18, 26].

Similar levels of total distribution volume (32.71 and 28.29 L) were found in both groups, which can be explained by a comparably low albumin concentration (26.6 and 26.8 g/L) and similar median weight of patients (80 and 100 kg). Despite achieving virtually identical C_{max} in all patients, the mean AUC_{24} (48.38 mg×h/L) in the group with preserved renal function was lower than in the group with impared renal function (71.50 mg×h/L), reflecting insufficient exposure. Even more significant differences ($P = 0.01$) between these categories of patients were found when

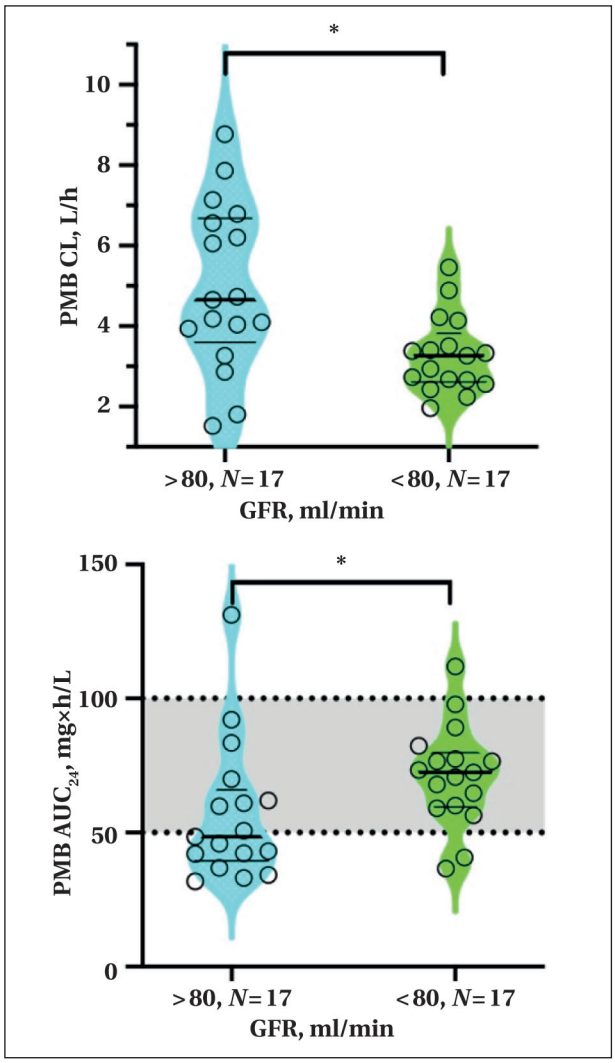

Fig. 4. Comparison of PMB clearance (CL) and exposure (AUC_{24}) depending on glomerular filtration rate (GFR).
Note. Data are presented as mean values ± standard deviations. * — $P < 0,05$.

comparing AUC values normalized to the daily dose (AUC_{24}/dose). Previously, researchers noted a similar fact: the area under the curve in patients with impared renal function was slightly higher vs patients with normal function (creatinine clearance ranged from 15 to 175 mL/min), although the statistical differences between the groups were insignificant [26].

The PK analyzis revealed that PMB clearance in patients with GFR < 80 mL/min was significantly lower ($P = 0.012$) compared to patients with preserved renal function (3.28 vs. 4.97 L/h), leading to significant increase in exposure ($P = 0.027$). In a PK study based on a two-compartment model, among 70 patients weighing 66–68 kg at PMB maintenance dose from 50 to 100 mg, clearance in patients with normal renal function was also significantly higher compared to patients with renal impairment (2.19 L/h compared to 1.58 L/h, respectively, $P < 0.001$). Moreover, AUC_{24} was significantly higher in patients with acute renal failure than in patients without renal impairment (108.66 ± 70.10 mg×h/L vs. 66.18 ± 34.79 mg×h/L; $P = 0.001$) [17].

Unlike most previous studies, including ours [20], focusing on PK estimatiom in patients with impaired renal filtration [4, 18], the present study makes emphasis on PMB inefficacy in patients with preserved renal function (GFR > 80 ml/min). Thus, failure to attain the minimum target AUC_{24} (50 mg×h/L) with the risk of treatment failure, was documented in 52.3% of patients (9/17) with preserved renal function, and only in 11.8% of patients (2/17) with impaired renal function. Higher AUC_{24} (> 100 mg×h/L) fraught with toxic manifestations, was documented in 2 patients — one from each group (5.9%). Therefore, in 58.8% (10/17) of patients with preserved renal function and in 17.6% (3/17) of patients with reduced filtration, AUC_{24} did not attain the target values (Fig. 3).

Impaired renal function comes with increased exposure to PMB due to decreased drug clearance and, conversely, renal hyperfiltration can lead to decreased antibiotic exposure. Thus, among patients ($N = 9$) with GFR > 120 ml/min, the median values of AUC_{24}, clearance, and volume of distribution were 45.76 mg×h/L (39.42; 76.73), 6.05 l/h (3.06; 6.78), and 40.24 L (26.6; 48.72), respectively. Similar data were obtained in patients with lower body weight [3]. In the present study, in more than half of the cases, patients with GFR > 80 ml/min had AUC_{24} values less than 50 mg×h/L. The target range of AUC_{24} = 50–100 mg×h/L was not attained, and most likely, such patients require dose adjustments, which is consistent with the observations of other researchers who call into question the concept of non-renal clearance of this antibiotic [18].

Limitations of the study. Preliminary registration of the prospective study on Clinical Trials platform and calculation of patient sample size were not done.

Conclusion

Thus, adherence to standard PMB dosing regimens, even taking into account the administration of a loading dose, does not always ensure that the target drug exposure is attained, which may be due to significant individual differences in PK in patients with sepsis.

It has been established that renal function significantly affects the pharmacokinetics of PMB in patients with sepsis: a decrease in renal function

increases drug exposure, while in preserved renal function drug exposure may be insufficient to achieve a therapeutic effect, especially in patients with increased body weight.

As a result, therapeutic drug monitoring is necessary to control therapy not only in patients with decreased renal function, but also in those with preserved renal clearance.

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Informativeness of Immunological Predictors of Prolonged and Chronic Critical Illness Outcome is Limited by Patient's Genotype

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Summary

The aim of the study was to determine the contribution of cellular immune system parameters and the *AQP4* (rs1058427) genetic polymorphism to the prognosis and outcome of patients with sequelae of severe brain injury (SBI), including patients who developed pneumonia.

Materials and Methods. The study included 464 intensive care unit (ICU) patients with prolonged or chronic critical illness (PCCI) admitted to the Federal Scientific and Clinical Center of Intensive Care Medicine and Rehabilitology (FSCCICMR) following SBI (strokes, traumatic brain and combined injuries, post-operative anoxic conditions, brain tumor surgery). Variants of the rs1058427 single-nucleotide polymorphism in the *AQP4* gene were detected in DNA isolated from whole blood with organic solvents and using genotyping with tetraprimer PCR followed by electrophoretic identification of the products.

Results. The entire cohort was divided into three groups of patients: those admitted without signs of pneumonia in the first 48 hours of hospitalization but who developed nosocomial pneumonia after 48 hours (group 1); admitted without signs of pneumonia, in whom no signs of pneumonia were detected throughout the hospitalization (group 2); with pneumonia diagnosed upon admission, which developed in the previous medical institution prior to transferring to the FSCCICMR (group 3). For the cohort combining groups 1 and 2 (admitted without signs of pneumonia), increased values of the neutrophil-to-lymphocyte ratio (NLR) (OR = 1.8, 95% CI: 1.1–3.9, $P = 0.0175$, χ^2, $N = 272$) and neutrophil count (OR = 2.1, 95% CI: 1.3–3.5, $P = 0.0038$, χ^2, $N = 272$) on the first day of hospitalization were associated with an increased risk of pneumonia. In the same cohort, elevated neutrophil counts (over 6×10^9/L) at admission significantly predicted adverse outcome, but only in the subgroup of patients with the *AQP4* rs1058427 GG major genotype (95% CI: 1.0–4.5, HR = 2.1, $P = 0.049$, log-rank test). In group 3 (patients with pneumonia diagnosed upon admission), a significant association with adverse outcome was found for both neutrophils and NLR (HR = 3.1, 95% CI: 1.3–6.9, $P = 0.019$, log-rank test, $N = 149$, and HR = 2.9, 95% CI: 1.3–6.6, $P = 0.026$, log-rank test, $N = 149$, respectively) in patients with *AQP4* GG genotype, not in alternative *AQP4* allele T carriers. Thus, the prognostic value of elevated neutrophil counts in patients with PCCI («immunophenotype») depends significantly on the genetic polymorphism of *AQP4*, a gene that controls the initiation of immune cell migration and is pathogenically significant for the development of the infectious process.

Conclusion. For patients with consequences of SBI in PCCI, an increase in neutrophil counts above 6×10^9/L upon hospitalization significantly predicts an adverse outcome only in patients homozygous for the *AQP4* rs1058427 G allele (GG genotype). The unique genetically restricted clinical and laboratory phenotype («gene-immunophenotype») could be considered in personalized critical care medicine as an example of a candidate predicting paradigm.

Keywords: chronic and prolonged critical illness; prognostic markers; nosocomial pneumonia; immune system cells; AQP4; genetic polymorphism; rs1058427

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

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Introduction

The number of patients in chronic or prolonged critical condition (CPCC) has doubled in recent decades and may double again in the next decade [1, 2]. These patients are also quite numerous among patients with the consequences of severe brain injury (SBI) requiring life support and hospitalized in intensive care units (ICUs) [3]. Persistent, long-term, prolonged critical conditions and chronical illness (PCCI), are defined as severe pathological conditions of patients requiring prolonged (at least 8–21 days) life support provided in intensive care units (ICUs). Current research has documented a growing number of such patients and a high incidence of in-hospital complications, accompanied by increased mortality. A separate group of patients with chronic brain injury includes those with sequelae of severe brain SBI resulting from trauma, hemorrhagic or ischemic stroke, prolonged brain surgery, intoxication, or hypoxia. These patients have survived the acute phase of the disease, ultimately resulting in a vegetative state or a state of low consciousness, and require prolonged intensive and rehabilitative treatment. The sequelae of SBI are accompanied by a cascade of pathological reactions not only in the brain (cerebral edema, cerebral hemodynamic disturbances, inflammatory complications, etc.). The cardiovascular, respiratory, digestive, water-balance, hormonal, and other systems are consistently and naturally involved in the pathological process. Impaired tissue nutrition and immune system function, changes in nutritional status, the development of hypotension, decreased tissue perfusion leading to hypoxia and multiple organ failure, the development of nosocomial infection, and the development of purulent-inflammatory complications complete the cycle of pathological reactions, increasing the likelihood of death [3].

Among the infectious complications developing with chronic coronary heart disease, pneumonia remains the most frequently diagnosed. The mortality rate of patients with pneumonia (etiologically unrelated to COVID-19) requiring intensive care unit (ICU) hospitalization is high, ranging from 13% to 30%. This high mortality rate may be due to frequent septic complications of severe pneumonia in ICU patients, resulting from persistent dysfunction of the innate and adaptive immune systems, which develops under conditions of prolonged bacterial infection and microbiota dysbiosis. Timely and appropriate use of antibiotics, high-tech methods of early diagnosis and treatment, and biomarkers help reduce mortality in severe pneumonia [4]. However, the informative value of the most commonly used biomarkers for pneumonia — procalcitonin and C-reactive protein — is only evident during treatment, when used to determine the duration of antibiotic use [4, 5]. Furthermore, personalized approaches to the effective treatment of critical conditions [1, 2, 6] require highly informative markers for disease prognosis. Such markers are unknown for the category of patients in the ICU. Therefore, the development of such markers for prognosticating the course and outcome of severe pneumonia in ICU patients is a pressing issue in critical care medicine.

The neutrophil-to-lymphocyte ratio (NLR) is a biomarker of the systemic inflammatory response used to assess the severity of the condition and predict outcome in various diseases: cerebrovascular accidents [7]; cardiovascular diseases [8]; bacterial, fungal infections and sepsis; community-acquired pneumonia; SARS-CoV-2 infection [9]; metabolic syndrome [10]; rheumatoid arthritis [11]; various types of cancer [12, 13]; decompensated liver cirrhosis [14]; severe trauma [15]. Quantitative NLR values are calculated based on a complete blood count by determining the ratio of the absolute number of neutrophils and lymphocytes in a unit of blood volume. The NLR value reflects a certain balance between the innate immune response (neutrophils) and adaptive immune reactions (lymphocytes) [16, 17]. Currently, the NLR is widely used as a reliable and readily available marker of immune system status in various infectious and non-infectious diseases. Critical illness and inflammation are characterized by a sharp increase in NLR values to 11–17, sometimes even above 30. Improved progression of sepsis and critical illness, as well as a reduced risk of mortality, are associated with a decrease in NLR values below 7. NLR helps differentiate more severe from milder illnesses. NLR is considered by many authors to be a popular and informatively promising biomarker of the balance of cellular immune systems, showing potential as a population predictor of adverse outcomes in various pathological conditions [16, 18–20]. Neutrophils are multifunctional granulocytic immune cells essential for providing first-line defense against invading pathogens. However, uncontrolled neutrophil activation can lead to severe, life-threatening complications caused by oxidative reactions, the production of oxygen and nitrogen radicals, cytokines, and other molecules that damage vascular endothelial cells. The binding of membrane-associated neutrophil structures to surface molecules of the vascular endothelium can disrupt the glycocalyx and compromise endothelial integrity. This contributes to tissue hypoperfusion, which is exacerbated by the penetration of activated neutrophils through the vascular wall [21, 22].

AQP4 is a protein that forms water channels in the cell membrane and is expressed in various tissues of the body, including the brain, kidneys, lungs, and the immune system. AQP4 expression promotes edema formation, determines the initial stages of immune cell migration, and maintains

the blood-brain barrier. *AQP4* plays a significant role in the development of pathological conditions. This protein controls the survival of neuronal cells and T cells. In vivo inhibition of AQP4 reduces the number of T lymphocytes in lymph nodes while simultaneously accumulating them in the liver. The 3' region of *AQP4* contains several functional single nucleotide substitutions. Polymorphisms in the 3' region of *AQP4* contribute to the development of cerebral edema after hemorrhagic stroke (*AQP4* T rs1058427) [23] and the outcome of traumatic brain injury (rs3763043) [24]. We previously demonstrated that the minor T allele of *AQP4* rs1058427, located in the 3' region of the gene, controls the favorable course and outcome of sepsis in a group of highly morbid intensive care unit (ICU) patients [24]. We also previously demonstrated that minor genotypes of *AQP4* rs1058427 increase the risk of developing pulmonary hypertension in patients with pleural empyema [25]. However, the possible influence of the 3' region polymorphism on the informativeness of cellular prognostic markers remained unclear. Since *AQP4* controls immune cell migration, and the *AQP4* rs1058427 polymorphism influences the prognosis of sepsis and the risk of complications in infectious diseases, we hypothesized that the prognostic value of cellular markers of the immune system may significantly depend on the *AQP4* genetic variant.

The aim of the study was to determine the contribution of cellular immune system parameters and the *AQP4* (rs1058427) genetic polymorphism to the prognosis and outcome of cerebral hemorrhage in patients with the consequences of severe brain injury, including the development of pneumonia.

Materials and Methods

We conducted an uncontrolled, prospective, observational, randomized study (decision of the Ethics Committee of the Federal Scientific and Clinical Center of Radiology and Radiology, Protocol No. 2.2.18 dated December 20, 2018). Patients were enrolled in the study between June 2018 and August 2021. According to our preliminary data, the mortality rate in patients with sequelae of cerebrovascular accidents admitted with pneumonia is approximately 13 percent, which was used to calculate the sample size. The formula for calculating the sample size was $\mathbf{n = (t2 \times P \times Q)/\triangle 2}$, where t is the critical value of the Student's *t*-test (at a significance level of 0.05, it is 1.96), $\triangle$ is the maximum permissible error (5%), **P** is the proportion of cases in which the studied characteristic is present (90), **Q** is the proportion of cases in which the studied parameter is not present (10), and **n** was 174. Considering that the frequency of the major genotype *AQP4* rs1058427 GG in the Moscow population is approximately 80 percent [24], we required at least 209 patients to recruit a sufficient number for calculations for patients with the major genotype. Half of the patients in our sample were admitted with pneumonia, therefore, to obtain the required number of patients with the major genotype *AQP4* rs1058427 GG, both with and without pneumonia, we required 418 patients in the cohort. Taking into account the possible lack of all data on the crucial for the study parameters (genotyping, course and outcome), it was decided to increase the planned cohort size by 10–12%. As a result, the entire cohort consisted of 467 patients. As a result of exclusion from the cohort due to incomplete data (loss of 2 blood samples for genotyping and lack of immunophenotype data during hospitalization (1 (patient), 464 ICU patients in PCCI as a consequence of cerebrovascular accidents (strokes, trauma, anoxic injuries, and brain tumors; Table 1) were included in the analysis. One hundred ninety-two patients in our sample were admitted with pneumonia. Upon admission, patients were assessed using the SOFA, Charlson, CIRS-G, and Glasgow Coma Scale scores. Allelic variants of *AQP4* rs1058427 were identified using tetraprimer polymerase chain reaction followed by electrophoretic separation and identification of stained products in the gel. The following primers were selected and synthesized at Evrogen LLC using the Primer-BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/):

AQP4 1 for 5`-TATTGGCAAAACTGGGGATT-3`

AQP4 2 for 5`-CCCAATCTCTGCTCTCTCAA-3`

AQP4 2 rev. 5`-GATTATCAACAAATGTCACGAGAAG-3`

AQP4 1 rev 5`-TGCAACCATGTTGTACCTTG-3`

The normal or not normal distribution of quantitative variables was assessed using the Shapiro–Wilk test. Normally distributed variables were described using mean values (*M*), standard deviations (*SD*), and 95% confidence intervals (95% CI). Non-normally distributed quantitative data were described using medians (*Me*), lower and upper quartiles (IQR). Normally distributed variables, provided that variances were equal, were compared across groups using the Student t-test. Non-normally distributed variables were compared using the Mann–Whitney *U*-test. Categorical data were described indicating absolute values, the ratios of which in the compared groups were analyzed using four-field contingency tables using the χ^2 test with Yates's correction for sample continuity (for $N > 100$) or Fisher's exact test (FET) for $N < 100$. Statistical analysis was performed using a two-sided test with a significance level of $P < 0.05$. The odds ratio (*OR*) with a 95% confidence interval (95% CI) and the relative risk (*RR*) with a 95% confidence interval (95% CI) were used as a quantitative measure of effect when comparing relative indicators. The log-rank test was performed for survival analysis according to Kaplan–Meier. The results were presented as the hazard ratio (HR) with a 95% confidence interval (CI). The

results of the Cox regression analysis were presented as the hazard ratio (HR) with a 95% confidence interval (CI). To predict the probability of an unfavorable outcome (mortality), the ROC curve method and logistic regression analysis were used. According to the literature, the normal value of the NLR is 0.78–3.53 [26]. Based on these considerations, we assumed that a NLR value above 4 may be a risk factor, since this value is outside the normal range. ROC analysis was used to determine the cutoff point for the content of lymphocytes and neutrophils. The cutoff point was established based on the highest value of the Youden index and corresponded to the most optimal ratio of sensitivity (*Se*) and specificity (*Sp*). Multivariate regression analysis was performed using the backward stepwise Wald method. Differences were considered significant at $P > 0.05$. Statistical analysis was performed using SPSS Statistics (version 27), MedCalc (version 11.6), and SigmaStat (version 3.5).

Results

Our study cohort included patients with SBT (Table 1).

Figure 1 presents patient mortality data by day of hospitalization.

Mortality in the entire cohort varied by the day of hospitalization (Fig. 1, *a*), with the maximum increases in the group of patients admitted without pneumonia in whom pneumonia was diagnosed during hospitalization (Fig. 1, *c*). In this group, mortality was characterized by a significant increase in mortality from days 10 to 30 of hospitalization, with peaks of up to 5 cases per day (Fig. 1, *c*), which could reflect the incidence of nosocomial pneumonia in patients predisposed to its development. In the group

Table 1. Demographic and clinical parameters of patients, *N* = 464.

Parameters	Values
Women, *N* (%)	186 (40%)
Age, *Me* (IQR)	58 (43–67)
SOFA scale score at admission, *Me* (IQR)	2 (1–3)
Diabetes mellitus, type II, *N* (%)	35 (7%)
Charlson Comorbidity Index (CCI), *Me* (IQR)	8 (6–10)
Comorbidity by Cumulative Rating Index Scale (CIRS) scale, *Me* (IQR)	14 (11–18)
Glasgow Coma Scale (SCG), *Me* (IQR)	14 (10–15)
Length of hospital stay, days, *Me* (IQR)	46 (30–66)
Lethality, *N* (%)	63 (14%)
Sequelae of ischemic stroke, *N* (%)	156 (34%)
Sequelae of hemorrhagic stroke, *N* (%)	114 (25%)
Sequelae of anoxic brain injury, *N* (%)	26 (6%)
Sequelae of traumatic brain injury, *N* (%)	99 (21%)
Brain tumors, *N* (%)	44 (9%)
Sequelae of severe COVID-19, *N* (%)	10 (2%)
Other*, *N* (%)	15 (3%)

Note. * — mixed genesis encephalopathy, consequences of previous neuroinfection, cerebrovascular disease, encephalomyelopolyneuropathy, other cerebrovascular diseases.

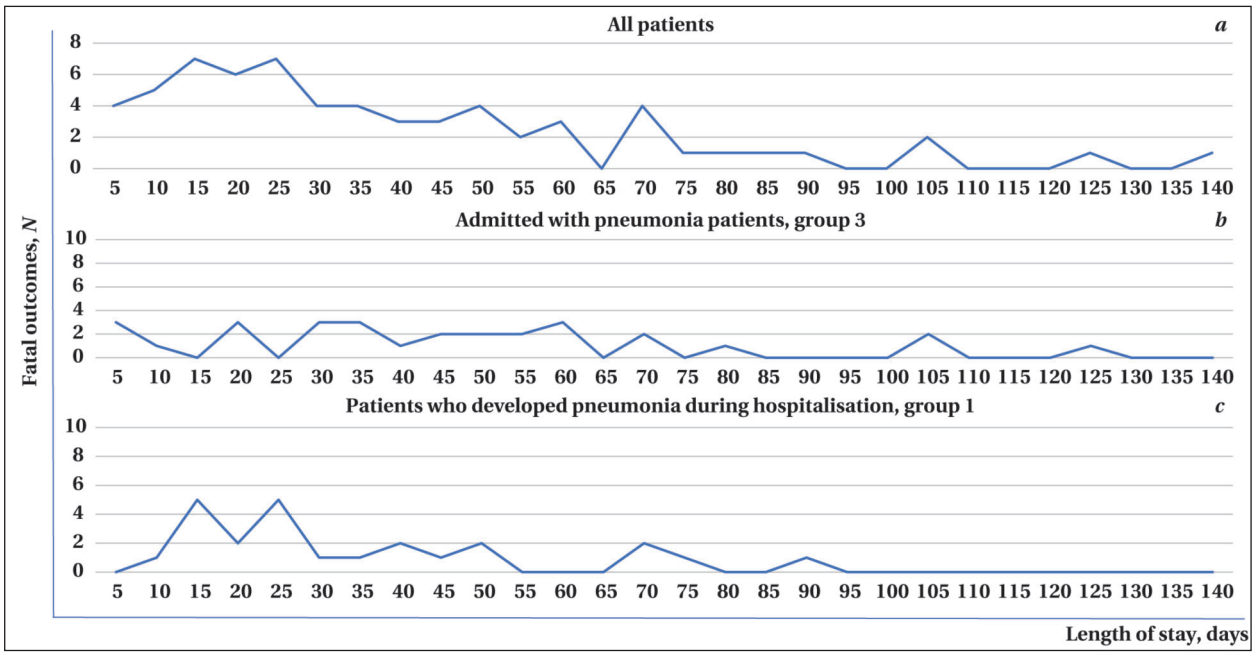

Fig. 1. Mortality of patients with sequelae of SBI by day of hospitalization.

of patients hospitalized with pneumonia (Fig. 1, *b*), peaks of higher mortality during the first month of hospitalization were virtually absent or were more smoothed out (not exceeding 3 patients per day).

It was of interest to identify early (on admittance) clinical and laboratory parameters associated with adverse outcomes in groups of patients with pneumonia and those predisposed to its development during the first weeks of hospitalization. As shown in Table 2, univariate logistic regression analysis of all patient data (N=464) revealed seven parameters out of nine that were significantly associated with mortality. Adjustment for the most common covariates in multivariate analysis revealed that the greatest contribution to mortality was attributed to two clinical parameters — the CCI and the GCS — as well as one integrated clinical and laboratory immunological parameter — the NLR (Table 2).

Calculations showed that a 1-point increase in CCI values was associated with a 26% increase in the odds of death, a 1-point decrease in the GCS was associated with a 15% increase in the odds of death, and a 1-point increase in the NLR was associated with a 3.8% increase in the odds of death. A similar analysis was conducted by dividing the entire cohort of patients into two groups, each differing in genotype: GG versus GT + TT, based on the results of genotyping the G and T alleles of *AQP4* rs1058427.

As can be seen from Table 3, in multivariate analysis, the same predictors of death persisted in patients with the GG genotype as in the entire sample (CCI, GCS, and NLR). For carriers of the *AQP4* T allele (GT and TT genotypes, N=92), similar information content was found only for the CCI values (Table 4). Using multivariate logistic regression, we found that for carriers of the minor T allele, both CCI and SOFA scores were significant predictors of mortality in ICU patients (Table 4).

Moreover, an increase in the CCI values by 1 point corresponded to an increase in the chance of death by 40.8% (corr. OR = 1.408; 95% CI = 1.077; 1.842), and an increase in the SOFA scale value by 1 point corresponded to an increase in the chance of death by 47% (corr. OR = 1.474; 95% CI = 1.043; 2.08). However, in contrast to patients with the *AQP4* GG genotype, in carriers of the T allele, the clinical and laboratory indicator of NLR was not a prognostic biomarker of death in either univariate or multivariate analyses (Table 4). Similar data confirming the selective prognostic value of immune

Table 2. Relationship between mortality rate and demographic indicators, assessment scales, and cellular parameters (logistic regression analysis).

Parameters	Univariate analysis		Multivariate analysis*		Exact Fisher test
	Odds Ratio (OR) (95% CI)	*P*	Corr. Odds Ratio (OR) (95% CI)	*P*	Odds Ratio (OR) (95% CI)
CCI	1.25(1.134–1.371)	<0.001	1.26 (1.138–1.394)	<0.001	2.2 (1.4–3.4)
GCS	0.852 (0.787–0.923)	<0.001	0.849 (0.78–0.93)	<0.001	3.0 (1.8–5.0)
CIRS	1.108 (1.056–1.164)	<0.001	—	—	2.4 (1.5–3.8)
SOFA	1.22 (1.101–1.351)	<0.001	—	—	3.0 (1.8–5.0)
NLR	1.22 (1.101–1.351)	<0.001	1.038 (1.003–1.074)	0.035	1.8 (1.1–2.8)
Neutrophils	1.058 (1.002–1.116)	0.042	—	—	2.4 (1.5–4.0)
Lymphocytes	0.928 (0.748–0.153)	0.5**	—	—	
Sex	0.84*** (0.484–1.456)	0.533**	—	—	
Age	1.034 (1.016–1.053)	<0.001	—	—	2.2 (1.4–3.4)

Note. Hear and in tables 3, 4: CCA — Charlson comorbidity index; GCS — Glasgow coma scale; NLR — neutrophil-to-lymphocyte ratio. Indicators not included in the multivariate regression analysis model: CIRS, SOFA, neutrophils, lymphocytes, gender, and age. * — Hosmer–Lemeshow goodness-of-fit test, P=0.799; Chi-square = 4.6; DF = 8, percentage of correctly classified cases 86.3. ** — regression is not statistically significant, P>0.05; *** — OR is presented for women

Table 3. Dependence of the incidence of death on demographic indicators, assessment scales and cellular parameters on the first day of hospitalization, patients with the *AQP4* rs1058427 GG genotype (logistic regression analysis).

Parameters	Univariate analysis		Multivariate analysis*		Exact Fisher test
	Odds Ratio (OR) (95% CI)	*P*	Corr. Odds Ratio (OR) (95% CI)	*P*	Odds Ratio (OR) (95% CI)
CCI	1.23 (1.1–1.37)	< 0.001	1.243 (1.112–1.388)	< 0.001	2.4 (1.5–4.0)
GCS	0.86 (0.79–0.937)	0.001	0.858 (0.78–0.94)	0.001	2.7 (1.6–4.8)
CIRS	1.101 (1.044–1.162)	< 0.001	—	—	2.1 (1.3–3.5)
SOFA	1.198 (1.075–1.34)	0.001	—	—	2.7 (1.6–4.8)
NLR	1.057 (1.02–1.096)	0.002	1.041 (1.002–1.08)	0.037	2.0 (1.1–3.3)
Neutrophils	1.11 (1.03–1.195)	0.005	—	—	2.5 (1.5–4.3)
Lymphocytes	0.96 (0.79–1.18)	0.7**	—	—	
Sex	0.76*** (0.4–1.4)	0.382**	—	—	
Age	1.032 (1.013–1.053)	0.001	—	—	2.5 (1.5–4.1)

Note. N=372. Parameters not included in the multivariate regression analysis model: CIRS, SOFA, neutrophils, lymphocytes, gender, age. * — Hosmer–Lemeshow goodness-of-fit test, p = 0.756; Chi-square = 5.02; DF = 8, percentage of correctly classified cases 85.9; ** — regression is not statistically significant; P>0.05; *** — OR is presented for women.

Table 4. Relationship between mortality rate and demographic indicators, assessment scales, and cellular parameters on the first day of hospitalization, patients with the *AQP4* rs1058427 GT and TT genotypes (logistic regression analysis).

Parameters	Univariate analysis		Multivariate analysis*		Exact Fisher test
	Odds Ratio (OR) (95% CI)	*P*	Corr. Odds Ratio (OR) (95% CI)	*P*	Odds Ratio (OR) (95% CI)
CCI	1.35 (1.06–1.7)	0.016	1.408 (1.077–1.842)	0.012	3.1 (1.0–9.9)
GCS	0.8 (0.63–1.012)	0.063**	—	—	
CIRS	1.14 (1.02–1.28)	0.022	—	—	2.5 (1.0–6.7)
SOFA	1.4 (1.02–1.9)	0.036	1.474 (1.043–2.081)	0.028	5.6 (1.3–24.5)
NLR	1.06 (0.97–1.15)	0.21**	—	—	
Neutrophils	1.01 (0.936–1.097)	0.748**	—	—	
Lymphocytes	0.59 (0.23–1.53)	0.278**	—	—	

Note. *N* = 92. Indicators not included in the multivariate regression analysis model: GCS, CIRS, NLR, neutrophils, and lymphocytes. * — Hosmer–Lemeshow goodness-of-fit test, *P* = 0.64; Chi-square = 6.08; DF = 8, percentage of correctly classified cases=85.6. ** — regression not statistically significant, *P* > 0.05.

Table 5. Correlation coefficients of predictors included in the multivariate logistic regression model.

		CCI, *r*, *P*	GCS, *r*, *P*	NLR, *r*, *P*	SOFA, *r*, *P*
All patients, *N* = 464	CCI	—	–0.031, 0.505	**0.130** (0.0056)**	—
	GCS	–0.031 (0.505)	—	**–0.236** (0.0001)**	—
	NLR	**0.130** (0.0056)**	**–0.236** (0.0001)**	—	—
AQP4 GG carriers, *N* = 372	CCI	—	–0.029 (0.58)	**0.167** (0.00135)**	—
	GCS	–0.029 (0.58)	—	**–0.210** (0.0001)**	—
	NLR	**0.167** (0.00135)**	**–0.210** (0.0001)**	—	—
AQP4 GT or TT carriers, *N* = 92	CCI	—	—	—	–0.005 (0.962)
	SOFA	–0.005 (0.962)	—	—	—

Note. ** — Spearman correlation is significant at the 0.01 level.

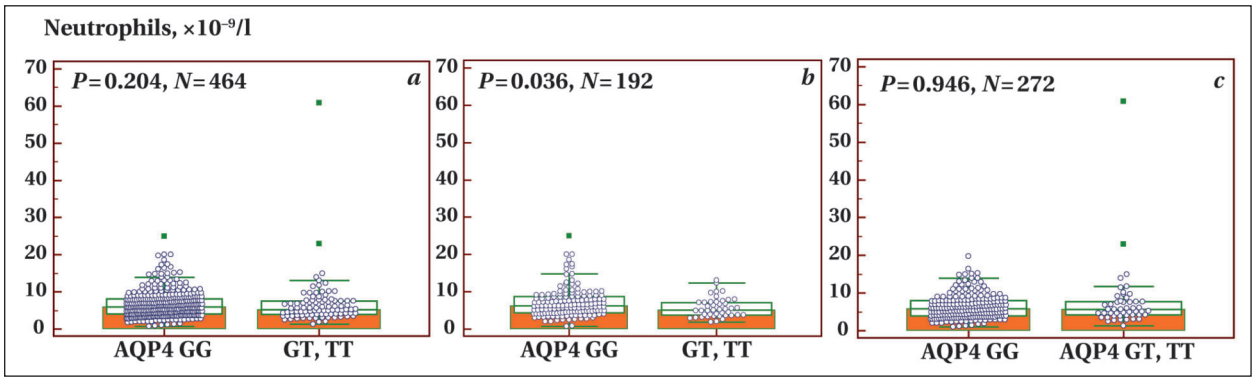

Fig. 2. Blood neutrophil counts in patients admitted to the PKC with different *AQP4* rs1058427 genotypes, depending on the presence of pneumonia on admission.
Note. *a* — all patients. The median (IQR) value for carriers of the major *AQP4* GG genotype was 6.0×10^{-9} per liter (4.1; 8.2), the mean (±σ) value was 6.6×10^{-9} per liter (±3.5). The IQR value for carriers of the minor *AQP4* GT and TT genotypes was 5.2×10^{-9} per liter (3.9; 7.6), and the mean (σ) value was 6.7×10^{-9} per liter (±6.6). *b* — patients admitted with pneumonia. The IQR for carriers of the major *AQP4* GG genotype was 6.2×10^{-9} per liter (4.4; 8.7), and the IQR for carriers of the minor *AQP4* GT and TT genotypes was 5.1×10^{-9} per liter (3.7; 7.2), while the IQR for carriers of the minor *AQP4* GT and TT genotypes was 5.7×10^{-9} per liter (±2.6). *c* — patients admitted without pneumonia. The IQR for carriers of the major *AQP4* GG genotype was 5.8×10^{-9} per liter (3.9; 8.0), and the IQR for carriers of the minor *AQP4* GT and TT genotypes was 6.3×10^{-9} per liter (±3.2). The IQR for carriers of the minor *AQP4* GT and TT genotypes was 5.6×10^{-9} per liter (4.2; 7.7), and the IQR for carriers of the minor *AQP4* σ genotype was 7.6×10^{-9} per liter (±8.6). The ordinate axis shows the neutrophil count, $\times10^{-9}$ per liter. *a, b, c* — Mann–Whitney test.

system cellular markers (neutrophils count and NLR values, but not lymphocytes count) in patients with the *AQP4* rs1058427 GG genotype were also obtained using Cox regression (Appendix). It should be noted that correlations between predictors included in the multivariate regression models remained weak or absent both when analyzing the entire patient cohort and when stratified by *AQP4* rs1058427 genotype (Table 5).

Neutrophil counts did not differ significantly between the overall sample and the subgroup of patients admitted to the hospital without pneumonia (Fig. 2, *a, c*). However, differences were found in the subgroup of patients admitted with pneumonia: carriers of the minor *AQP4* T allele had lower neutrophil counts (median values 6.2 (GG) and 5.1×10^{9}/L (GT, TT), *P* = 0.036, Mann–Whitney test, *N* = 192, Fig. 2, *b*).

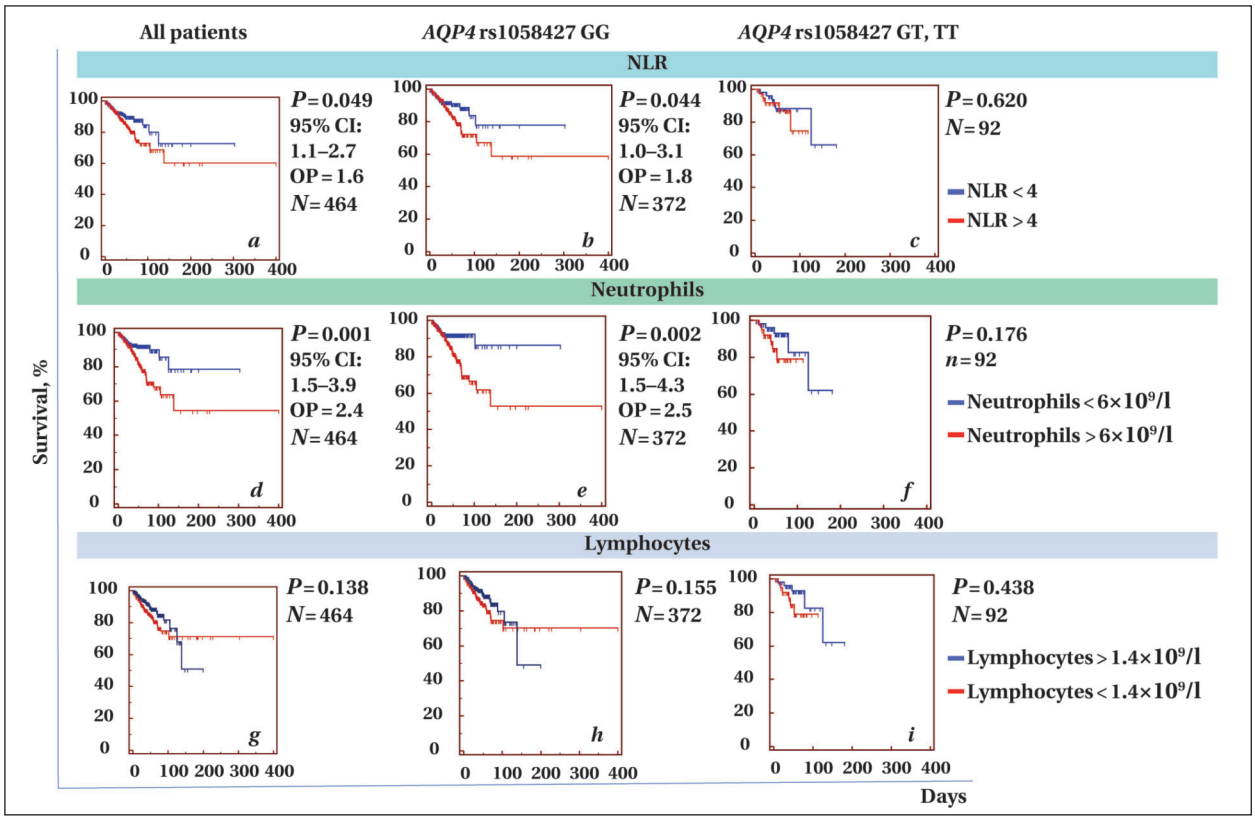

Fig. 3. Association of NL, neutrophil, and lymphocyte counts with outcome (entire cohort of patients in the PCCI).
Note. Here and in Fig. 4–5: *a* — NLR values, all patients; *b* — NLR values, patients with the *AQP4* rs1058427 GG genotype; *c* — NLR values, patients with the *AQP4* rs1058427 GT, TT genotypes; *d* — neutrophil count, all patients; *e* — neutrophil count, patients with the *AQP4* rs1058427 GG genotype; *f* — neutrophil count, patients with the *AQP4* rs1058427 GT, TT genotypes; *g* — lymphocyte count, all patients of the cohort; *h*— lymphocyte count, patients with the *AQP4* rs1058427 GG genotype; *i* — lymphocyte count, patients with the *AQP4*.

In subsequent analysis, using the log-rank test, we found that the value of NLR on the first day of hospitalization predicted outcome for patients with all *AQP4* rs1058427 genotypes ($P=0.049$, log-rank test, 95% CI: 1.1–2.7, HR = 1.6, $N=464$, Fig. 3, *a*). For patients with the major AQP4 rs1058427 GG genotype, the association remained ($P=0.044$, 95% CI: 1.0–3.1, HR = 1.8, $N=372$, Fig. 3, *b*). However, in the subgroup of patients carrying the minor T allele (GT and TT genotypes), no trend toward an association between the NLR value and adverse outcome was observed ($P=0.621$, $N=92$, Fig. 3, *c*).

Over 40% of patients in our sample were admitted to the hospital with pneumonia. The frequencies of the AQP4 rs1058427 genotypes did not differ in patients admitted with pneumonia (GG — 78%. GT — 21%, TT — 1%, consistent with the Hardy-Weinberg law, P = 0.657, $\chi^2=0.2$, $N=192$) and without pneumonia (GG — 82%. GT — 16%, TT — 2%, consistent with the Hardy–Weinberg law, $P=0.116$, $\chi^2=2.5$, $N=272$). For the group of patients admitted without pneumonia, the value of NLR as a prognostic marker was not statistically significant ($P=0.240$, $N=92$, Fig. 4, *a*). When subgroups of patients with different *AQP4* rs1058427 genotypes were analyzed separately, no patterns were found (Fig. 4, *b*, *c*). Only an increased neutrophil count significantly predicted an unfavorable outcome for patients admitted without pneumonia ($P=0.010$, log-rank test, 95% CI: 1.5–4.9, HR = 2.5, $N=272$, Fig. 4, *d*). However, when the patient genotype was taken into account, the association between an increased neutrophil count and an unfavorable prognosis was significant only for patients with the major *AQP4* rs1058427 GG genotype ($P=0.049$, log-rank test, 95% CI: 1.0–4.5, HR = 2.1, $N=223$, Fig. 4, *e*). For minor allele carriers, no such association was observed ($P=0.100$, $N=49$, Fig. 3, *f*). Circulating lymphocyte counts at admission for the subgroup of patients admitted without pneumonia were not associated with outcome regardless of *AQP4* rs1058427 genotypes (Fig. 4, *g*, *h*, *i*).

It should be concluded that a significant association with adverse outcomes for the immunophenotype of elevated neutrophil counts (over 6×10^9/L) in patients admitted to the PCC without signs of pneumonia is observed only when combined with the major *AQP4* rs1058427 GG genotype. Thus, the presence of a combined «geno-immunophenotype» such as the *AQP4* rs1058427 GG genotype and a

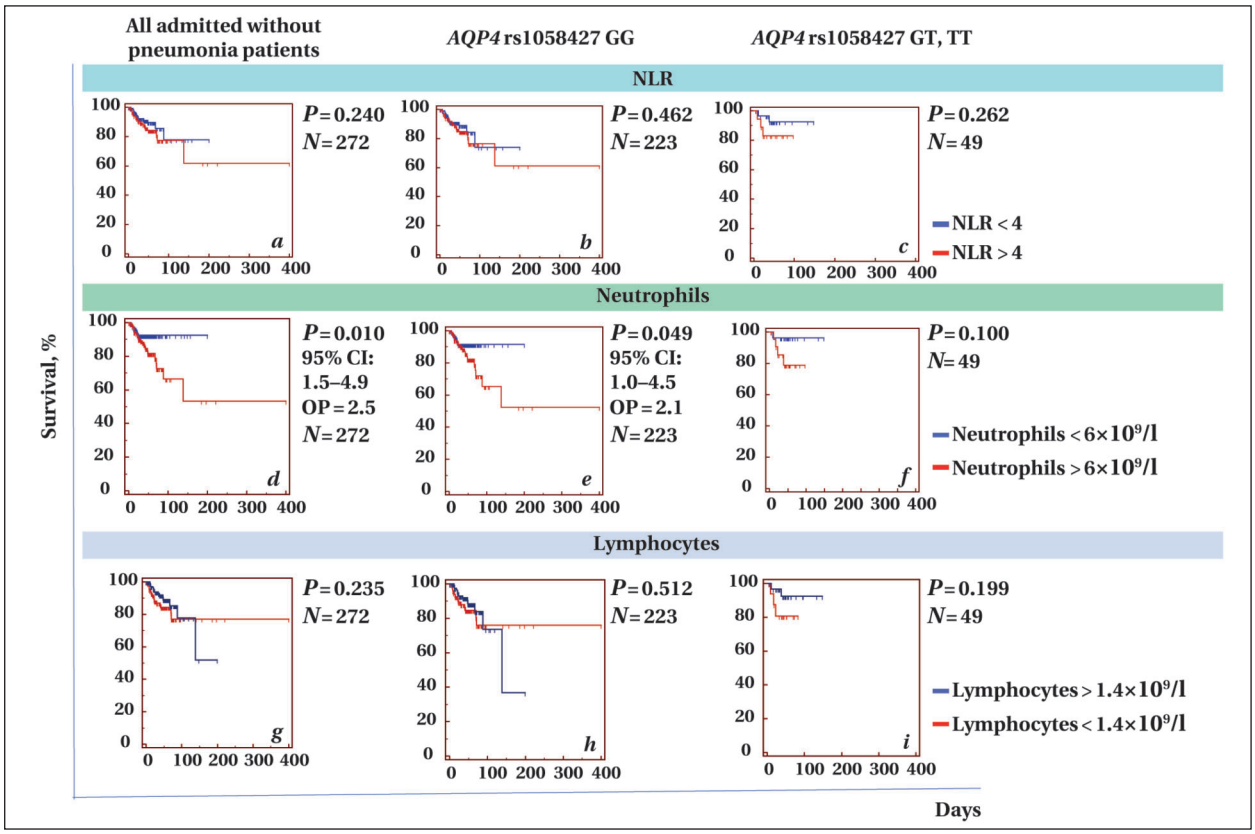

Fig. 4. Association of neutrophil counts, neutrophil counts, and lymphocyte counts with outcome in patients admitted without pneumonia.

neutrophil count exceeding 6×10⁹/L in a patient without signs of pneumonia upon hospitalization determines a high risk of mortality. Another group of patients was further analyzed — those diagnosed with pneumonia upon admission (within the first 48 hours of hospitalization). In the group of patients of all genotypes admitted with pneumonia, a statistically insignificant trend towards increased mortality was revealed in patients whose NLR values exceeded 4 on the day of admission ($P = 0.096$, log-rank test, Fig. 5, *a*). Statistically significant association between increased NLR value and mortality in this group of patients was observed only in carriers of the homozygous G allele ($P = 0.026$, log-rank test, HR = 2.9, 95% CI: 1.3–6.6, $N = 149$, Fig. 5, *b*). In the subgroup of carriers of the minor T allele, no trend towards increased mortality was even revealed with an NLR value of more than 4 ($P = 0.65$, $N = 43$, Fig. 5, *c*).

An increase in the blood neutrophil count above 6×10⁹/L predicted an unfavorable outcome for all patients admitted with pneumonia ($P = 0.034$, log-rank test, HR = 2.3, 95% CI: 1.1–4.7, $N = 192$, Fig. 5, *d*). However, a separate subgroup analysis of patients with different *AQP4* rs1058427 genotypes revealed a similar pattern to that found for ANP: differences were significant only for patients with the *AQP4* rs1058427 GG genotype ($P = 0.019$, log-rank test, HR = 3.1, 95% CI: 1.3–6.9, $N = 149$, Fig. 5, *e*). For patients with alternative genotypes GT and TT, prognosis did not differ depending on the blood neutrophil count ($P = 0.760$, $N = 43$, Fig. 4, *f*). A reduced lymphocyte count in our sample was not associated with an unfavorable prognosis for either the subgroups with the *AQP4* rs1058427 GG genotype ($P = 0.167$, $N = 149$, Fig. 5, *h*) or for the subgroup of carriers of the minor T allele ($P = 0.649$, $N = 43$, Fig. 5, *i*).

Thus, both the increased blood neutrophil count and its secondary marker — an elevated NLR value during hospitalization of patients with pneumonia — indicate an increased risk of an unfavorable disease course not in any patients, but only in patients with the *AQP4* rs1058427 GG genotype. It should be assumed that, as in the previous group of patients (hospitalized without signs of pneumonia), the *AQP4* rs1058427 allele complements the prognostic value of the immunophenotype (in the presence of pneumonia, these are two related indicators, NLR and an elevated neutrophil count), forming in concert a unique, combined gene-immunophenotype.

Discussion

A logistic regression analysis revealed that, of simple laboratory parameters, only elevated neutrophil counts (6×10⁹/L) and derived marker values — NLR (exceeding the norm, i. e., more than 4) —

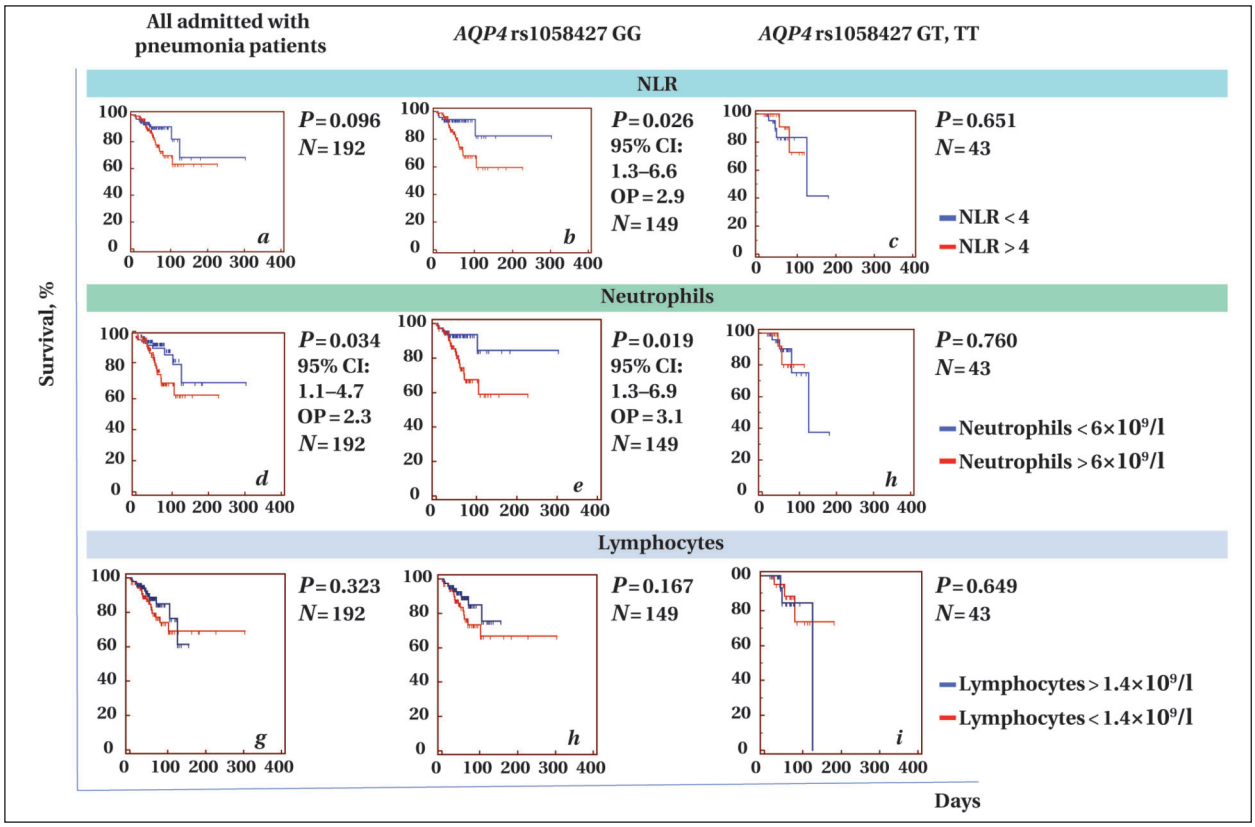

Fig. 5. Association of NLR, neutrophil and lymphocyte counts with outcome in patients admitted with pneumonia.

were associated with an unfavorable outcome in patients with the consequences of SBI diagnosed with PCCI (Table 2). A log-rank test confirmed the unfavorable prognosis when analyzing the entire cohort of patients using both parameters separately as prognostic biomarkers (Fig. 3). However, further analysis dividing the entire cohort into genetically distinct subgroups revealed limitations in the prognostic value of cellular immune parameters associated with genetic variability in patients for the *AQP4* gene alleles.

Earlier, it has been shown that the major allele of *AQP4* rs1058427 is associated with the development of edema after hemorrhagic stroke [23] and the outcome of sepsis in highly morbid patients [24]. In current study, in another cohort of patients (patients with the consequences of cerebrovascular accidents, admitted to the hospital), for the first time we demonstrated the contribution of the same *AQP4* genetic region to the formation of a complex gene-immunophenotype of patients with a high risk of unfavorable outcome. These were the patients with the *AQP4* GG genotype and an immunophenotype characterized by an increased number of neutrophils or the NLR value who had a significantly higher risk of death. For patients with the major genotype *AQP4* GG, NLR values above 4 had predictive informativeness regardless of the diagnosis of pneumonia (Tables 3–5). Perhaps, this reflects the differentiated effect of the *AQP4* gene on the processes of migration of neutrophils from the bone marrow and lymphocytes from the lymph nodes, expressed during activation of the infectious process. In patients with the major genotype *AQP4* admitted with pneumonia, the number of neutrophils was higher (Fig. 2), which may reflect the causative reason for the association of both biomarkers with mortality. It can be assumed that the presence of the minor T allele in the regulatory 3`-untranslated region of the *AQP4* gene rs1058427 in patients with PCCI determines the reduced level of *AQP4* gene expression in neutrophil precursor cells and, as a consequence, their lower level of migration into the bloodstream.

Populations of immature neutrophils contain subpopulations of granulocytic myeloid-derived suppressor cells (G-MDSCs) [27, 28], and with a relatively reduced content of neutrophils in circulation, a decrease in the number of G-MDSCs should be expected. In this case, the reduced potential of proinflammatory and antibacterial activity of the entire neutrophil mass as key cells of innate immunity can be compensated by improved development of antibacterial adaptive immunity due to the expected reduced number of G-MDSCs, which are known suppressors of the functional activity of

adaptive immune cells [27, 28]. On the contrary, the association we identified between the *AQP4* GG genotype and an increased number of neutrophils in the bloodstream in patients with pneumonia may lead to a higher content of circulating G-MDSCs in the blood and, consequently, to a greater manifestation of their functional activity. As a result, the antibacterial immune system may become weaker, and the risk of a fatal outcome in pneumonia in PCCI patients may increase. NLR reflects the balance between innate immunity and adaptive immunity. Increased NLR values may indicate the preferential circulation of the proinflammatory innate immune cells and/or decreased number of lymphocytes. These alterations may reflect the immune potential to contribute to both neutrophil-induced tissue damage at sites of localized infection (including abscess formation [29]) and decreasing the antibacterial activity mediated by T and B cells of adaptive immunity.

We believe that, in addition to increased blood neutrophil counts and NLR values, a key component of the immunophenotype associated with an unfavorable course and outcome of pathological conditions, including PCCI, may be an increased number of G-MDSCs, which are capable of being activated by bacterial endotoxins [30] and are associated with an unfavorable outcome of sepsis [31].

An increase in blood neutrophil counts and, consequently, an increase in NLR values are observed in various conditions for which inflammation is a key factor in pathogenesis: bacterial and fungal infections, acute stroke, myocardial infarction, atherosclerosis, trauma, tumors, postoperative complications — that is, in conditions characterized by tissue damage, activation of the systemic inflammatory response, and the development of organ failure [15, 16, 18–20]. Increased neutrophil activation markers on the first day of hospitalization for COVID-19 predicted subsequent transfer of patients to the intensive care unit (ICU) and an unfavorable course of the disease [22].

Lower NLR values are generally associated with a favorable prognosis for pathological conditions, reflecting a favorable immune balance [31]. Assessing this balance by determining NLR helps predicting the outcome in mechanical ventilation-associated pneumonia patients [31, 32], as well as in pneumonia developing after severe stroke. The latter is characterized by immunosuppression resulting from the enhancement of two processes: apoptotic lymphocyte death and the release of maturing neutrophils from the bone marrow into the bloodstream due to stimulation of granulocyte maturation by growth factors [33, 7]. Ventilator-associated pneumonia is one of the most severe complications in patients with traumatic brain injury (TBI) and is considered a risk factor for adverse outcomes [35]. Elevated NLR values predict adverse outcomes in patients with TBI and correlate with lower GCS scores [36, 37], which is consistent with our results.

Our findings suggest that testing a patient's genetic polymorphism (in particular, *AQP4*) may be useful for more targeted use of NLR and neutrophil count as a biomarker immunophenotype characterizing existing immune system imbalance. The reasons for the selective prognostic value of elevated NLR and/or neutrophil counts only in patients with the *AQP4* rs1058427 GG genotype of PCCI patients may relate to the increased functional significance of the AQP4 molecule for immune system cells, neuroglia, epithelium, and endothelium — key «players» and cellular targets in various pathological conditions, including infectious and inflammatory processes [38–40].

These data substantiate the advisability of expanding the concept of immunophenotypes to «predictive geno-immunophenotypes», more specifically, taking into account the genetic variability of patients, characterizing their high risk of a fatal outcome from PCCI. The search for and application of such combined predictive immunophenotypes/genotypes as prognostic biomarkers useful for early stratification of patients into risk groups will complement the existing principles of phenotyping critically ill patients [41–45] through genotyping in order to improve treatment outcomes in the format of personalized critical care medicine.

Conclusion

For patients in PCCI with sequelae of SBI, a neutrophil count above 6×10^9/L upon hospitalization significantly predicts an adverse outcome only in patients homozygous for the *AQP4* rs1058427 G allele (GG genotype carriers). It is suggested to consider the polymorphic genetic markers as a conceptual tool and a biomarker source complementing existing principles of clinical and laboratory phenotyping of patients in predictive critical care medicine («predictive immunophenotype/genotype for critical illness»).

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Приложение

Таблица. Результаты анализа выживаемости, проведенного с помощью регрессии Кокса на основе значений клеточных маркеров в 1-й день госпитализации.

Показатели	Все пациенты			*AQP4* rs1058427 GG			*AQP4* rs1058427 GT, TT
	P	**ОР**	**95% ДИ**	***P***	**ОР**	**95% ДИ**	***P***
Лимфоциты	0.62				0.84		0.16
Нейтрофилы	**0.006**	1.0	1.0–1.1	**0.032**	1.1	1.0–1.2	0.32
ОНЛ	**0.0001**	1.0	1.0–1.1	**0.0001**	1.0	1.0–1.1	0.22

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Casual Relationship between the GUT Microbiota Dysbiosis, Intestinal Motility, and Development of Protein-Energy Malnutrition in Patients in a Chronic Critical State after Severe Brain Damage

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Summary

Protein-energy malnutrition (PEM) remains one of the most pressing issues in patients with severe traumatic brain injury in intensive care units (ICUs), as it is highly prevalent, difficult to manage, and its causes are not fully understood.

The aim of the study was to assess the influence of gut microbial imbalance and gastrointestinal motility on the development of malnutrition in patients in a chronic critical state and severe brain damage.

Materials and methods. A single-center prospective observational study included 31 patients (median age 52 years; 68% males) aged 18–74 years with traumatic brain injury or stroke requiring ICU stay for more than 5 days and enteral tube feeding. Patients with diabetes mellitus, acute multiple organ failure (MOF), shock, implanted devices, or tracheoesophageal fistula were excluded. Nutritional status was assessed at baseline and on Day 20 using the Russian malnutrition scale and the Global Leadership Initiative on Malnutrition (GLIM) criteria. Additionally, clinical outcomes, anthropometric data, gastrointestinal biomarkers, gut microbiota composition, electrogastroenterography (EGEG) and functional scales parameters were recorded.

Results. Moderate and severe malnutrition according to the GLIM criteria was found at baseline in 29.1% of patients, and in 27.7% of patients on Day 20 ($P=0.9$), while according to the Russian scale these numbers were 61.3% and 78.6%, respectively ($P=0.8$). Dynamics of clinical scales, functional indicators, and gastrointestinal biomarkers during the follow-up revealed no clinically significant changes. Significant and persistent deviations 2 from reference values in gut microbiota composition (decrease in the content of *E. coli*, $P=0.026$; increase in *Enterobacter* spp., $P=0.020$) and EGEG parameters were recorded at both evaluation time-points. Identified PEM was also associated with impaired gastrointestinal motility.

Conclusion. The data indicate a statistically significant relationship between PEM, changes in the gut microbiota and gastrointestinal motility, which confirms the important role of these factors in PEM pathogenesis in patients with severe brain damage in a chronic critical state.

Keywords: protein-energy malnutrition; ICU; microbiota; electrogastography; stroke; traumatic brain injury

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

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Introduction

Severe brain damage, whether it is traumatic brain injury (TBI) or acute cerebrovascular accident (CVA), is a serious pathology that can cause severe neurological deficits and prolonged/chronic critical conditions, requiring long-term neurorehabilitation [1–3]. Protein-energy deficiency (or nutritional deficiency, or malnutrition — PEM) is a widespread and clinically significant complication in patients with severe TBI, associated with adverse outcomes and increased mortality [4, 5]. This is a multifactorial condition resulting from interaction of various pathogenetic factors that disrupt the regulation of metabolic processes [6]. Gut dysfunction in critical conditions is characterized by a series of pathological responses, including increased gut permeability syndrome, gut microbiota dysbiosis, and altered gastrointestinal tract (GIT) motility [7, 8].

The severity of the disease, high energy consumption, intense catabolism, and gastrointestinal dysfunction make enteral nutrition insufficient to achieve energy and substrate balance in such patients [9]. There is growing evidence that disruption of gut microbiota balance affects other organs and contributes to progression of critical diseases [10, 11]. We have earlier established the association between the neurological status of patients with severe brain damage and considerable alterations in the composition and metabolic activity of gut microbiota [12]. It has been shown that a disruption in the intestinal barrier function, manifested in increased intestinal permeability, is associated with a high risk of developing sepsis and multiple organ failure [13].

Gastrointestinal motility disorders are a common and significant complication in critically ill patients, leading to various adverse consequences interfering with recovery of vital functions [14]. Gastrointestinal motility dysfunction contributes to establishing PEM, therefore can be considered as a promising therapeutic target in patients with disrupted gut-brain axis, which is common in severe brain injuries [15]. Electrogastroenterography (EGEG) is a modern non-invasive diagnostic modality based on registration of gastrointestinal tract myoelectric activity. This modality allows for quantification of electrical activity, and demonstrates high efficiency in assessing neurological dysfunction [16–19]. Despite the available data, the relationship between various manifestations of intestinal failure syndrome and nutritional status requires further study. The aim of the study was to comprehensively assess gastrointestinal dysfunction and its relationship with the degree of malnutrition in patients in a chronic critical condition after severe brain damage.

Materials and Methods

The study outline. A single-center prospective observational clinical study was conducted. The study protocol was approved by the local ethics committee of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitation (No. 2/24/6 dated June 18, 2024) and registered on the ClinicalTrials.gov platform (NCT06545825). The study was initiated on September 9, 2024 as a pilot, exploratory, and observational, therefore no formal calculation of sample size or power was performed. The study was planned and designed in accordance with the principles of the Declaration of Helsinki [20]. The protocol was developed in accordance with the recommendations of SPIRIT 2013 [21]. The manuscript was prepared in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for reporting observational studies [20]. The STROBE checklist is presented in the supplementary table A1 (the supplementary materials for the article are published in Mendeley Data, doi: 10.17632/76hzzg54t7.1; https://data.mendeley.com/datasets/76hzzg54t7/1).

Selection criteria and study population. In the period from September to November 2024, all patients admitted to the Federal Scientific and Clinical Center for Resuscitation and Rehabilitation (FSCC RR) were screened for eligibility criteria. The inclusion criteria were: age between 18 and 74 years; presence of a traumatic brain injury or stroke; duration of stay in the intensive care unit exceeding 5 days at the time of screening (prolonged or chronic critical condition) [22]; and optionality of enteral nutrition.

All patients were transferred from other medical facilities and had previously received treatment in the ICU, including antibiotic therapy with one or more antimicrobial drugs.

The exclusion criteria included the following conditions: diabetes mellitus, acute kidney injury, acute liver failure or shock of any etiology; the presence of cardiac implantable electronic devices (CIEDs) or a neurostimulator, or a tracheoesophageal fistula. Patients who needed to maintain a positive end-expiratory pressure of more than 12 mBar were also excluded.

If all the eligibility criteria were met, a voluntary written informed consent was obtained from the participants, or, if this was not possible, the decision to include the patient was made by a medical council of the institution in accordance with regulatory requirements.

Data collection and outcomes. For each patient included in the study, an electronic individual registration form was completed based on a comprehensive clinical assessment. The collected data included demographic characteristics (age, gender, height, and body weight), medical history, informa-

tion about concomitant pathology and concomitant therapy, as well as hospital outcomes: mortality, need for mechanical ventilation (MV), duration of MV, use of vasoactive drugs, duration of enteral nutrition, and presence of specified complications.

Nutritional status was assessed on Day 0 (at inclusion), and on Day 20 according to the Global Leadership Initiative on Malnutrition (GLIM) criteria [23] and the national Russian malnutrition assessment scale (Supplementary Table A2). The risk of malnutrition was assessed using the Prognostic Nutritional Index (PNI), calculated as follows: [10 × serum albumin (g/dL)] + [0.005 × absolute lymphocyte count (in mm³)] [24].

On Days 0 and 20, vital signs and anthropometric data were recorded. The neurological and functional status was assessed using the modified Rankin Scale (mRS), the Barthel Index, and the Functional Independence Measure (FIM). Evaluated gut excretion biomarkers included nitrogen excretion, fecal zonulin concentration, and fecal α-1-antitrypsin. Also gut microbiota composition and electrogastroenterography (EGEG) parameters were examined.

Microbiota assessment. Fecal samples were collected in disposable sterile containers. The containers were immediately transported to the laboratory and frozen. Gut microbiota composition was analyzed using the real time PCR (qPCR) and the Kolonoflor-16 test systems (AlfaLab LLC, St. Petersburg, Russia) in accordance with the manufacturer's instructions. Composition of gut microflora was evaluated using the DT-prime amplifier (DNA Technology LLC, Moscow, Russia).

Electrogastroenterography. EGEG was performed on an empty stomach using the Gastroscan-GEM system (Istock-System JSC, Russia) with the Gastro-Scan software version 20.8. Spectral analysis included the coefficient of rhythm calculation (rate rhythm Kritm = peak frequency / dominant power), relative segmental power (P (i) / PS), and intersegmental propagation coefficient (P (i) / P (i+1)) [19, 25].

Statistical analysis. The data were processed statistically using the IBM SPSS Statistics program for Windows, version 29.0 (IBM Corp., Armonk, NY, USA). Quantitative (continuous) variables were summarized as the median and interquartile range (IQR), and categorical variables were presented as absolute values and percentages. The data distribution type was assessed using the Shapiro–Wilk test and histogram analysis.

Quantitative variables whose distribution did not follow a normal distribution were compared using the Mann–Whitney test. For paired quantitative data, the Wilcoxon paired rank test was used. Categorical variables were compared using the χ^2 test or the Fisher exact test (depending on the applicability conditions); for paired binary data, the McNemar test was used. The Spearman rank correlation coefficient was used for correlation analysis. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

The Python programming language (version 3.10) and the Matplotlib (version 3.10) and Pandas (version 2.2.3) libraries were used for data visualization.

Results

Patients' characteristics. Between September and November 2024, 39 patients admitted to the ICU were examined for compliance with the inclusion criteria. Eight patients were excluded based on the exclusion criteria, therefore 31 patients (21 men) with a median age of 52 years (IQR 40; 68) were included into final analysis. The flowchart of patient selection is presented in Fig. 1.

The baseline demographic and clinical characteristics, including information on comorbidities, concomitant therapy, and outcomes related to ICU stay, are presented in Table 1. Nine patients were diagnosed with TBI, 21 patients presented with ischemic or hemorrhagic stroke, and 1 patient had a combination of TBI and stroke. Arterial hypertension was found in 22 patients (71%).

All patients required respiratory support (low- or high-flow oxygenation, or mechanical ventilation in various modes). All patients also continued on antimicrobial therapy, either prescribed earlier or modified. Patients' neurological status was characterized by varying degrees of impaired consciousness, cognitive and speech disorders, and severe dysphagia, which necessitated feeding through a nasogastric tube or gastrostomy.

During the follow-up period, one patient died, another was transferred to another medical facility before the study was completed. Twenty patients (65%) required mechanical ventilation, with a median duration of 17 days. Six patients (19%) received

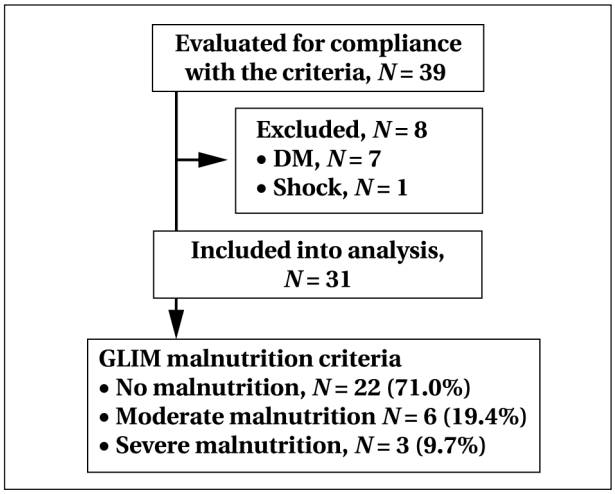

Fig. 1. The flowchart of patient selection for the study.

vasopressor or inotropic support. Seven patients (22.6%) developed sepsis according to the Sepsis-3 criteria, and eight patients (25.8%) developed multiple organ failure.

PEM characteristics within the follow-up. At baseline, the Russian scale of malnutrition assessment showed moderate and severe degree PEM in 19 patients (61.3%), and by Day 20, the proportion of such patients tended to increase (78.6%, $P=0.8$; Table 2).

Baseline assessment by the GLIM criteria identified moderate and severe malnutrition in 9 patients (29.1%). By Day 20, the overall proportion of patients with moderate and severe malnutrition did not significantly change (27.7%; $P=0.9$; Table 2).

Changes in anthropometric and clinical parameters. Anthropometric data did not change over time, except for a decrease in the mid-upper arm circumference (median: 24.5 cm vs. 23.3 cm; $P=0.004$, Table 2).

Clinical scales, including the mRS, Barthel Index, and FIM, also did not show any differences (Table 2). No statistically significant changes in nitrogen excretion and fecal α-1-antitrypsin concentration were observed during the observation period ($P=0.4$ and $P=0.9$, respectively). Despite the statistically significant deviation in fecal zonulin concentration to 11.5 ng/mL from a median value of 7,5 ng/ml ($P=0.028$), it remained within the reference range.

No statistically significant changes in colonic microbiota composition were detected (Fig. 2, Additional Table A3). However, there were significant deviations from the reference values in both assessments. More than 50% of patients had an increased titer of pathogenic bacteria, including *Klebsiella pneumoniae, Proteus vulgaris / mirabilis, Enterobacter* spp., and *Acinetobacter* spp. and an increase in the ratio of *Bacteroides* spp. / *Faecalibacterium prausnitzii*. A decrease in the content of «beneficial» bacteria, including *Lactobacillus* spp., *Bifidobacterium* spp., *Faecalibacterium prausnitzii, Blautia* spp., *Eubacterium rectale*, and *Roseburia inulinivorans* was documented in greater part of the cohort.

A significant proportion (more than 70% for some parameters) of patients showed significant deviations from the physiological reference values of the EGEG parameters in both assessments (Fig. 2, Additional Table A3). Most of them had gastrointestinal dyscoordination: increased absolute rhythmic activity in the upper parts (stomach, duodenum) against decreased activity in the lower parts (jejunum, ileum, colon). Simultaneously, noticeable changes in the stomach and duodenum, ileum, and colon contractile patterns were revealed. Over time, these parameters tended to normalize.

PEM associated changes. A comparative analysis of patients with normal nutritional status and patients with moderate and severe protein-energy malnutrition according to the GLIM criteria showed statistically significantly lower values of anthropometric parameters in patients with PEM, including BMI and the triceps skinfold thickness on Day 20 ($P=0.006$ and $P=0.015$, respectively). In ad-

Table 1. Patients' baseline characteristics, clinical features, concomitant therapies and clinical outcomes.

Parameter	Values
Total number of patients	$N=31$
Gender	
M	21 (68%)
F	10 (32%)
Age, years	52 (IQR 40; 68)
BMI, kg/m²	26.0 (IQR 21.6; 29.4)
Occurrence rates of various pathologies on admission	
Stroke	22 (71.0%)
Arterial hypertension	22 (71.0%)
Traumatic brain injury	10 (32.3%)
Pleural effusion	5 (16.1%)
Sepsis	4 (12.9%)
Congestive heart failure (NYHA II, III, IV)	3 (9.7%)
Atrial fibrillation	2 (6.5%)
Myocardial infarction	2 (6.5%)
Chronic kidney disease (stage C3b)	1 (3.2%)
Renal replacement therapy	1 (3.2%)
Drug use for concomitant therapy	
Anticoagulants	27 (87.1%)
Antibacterial drugs	22 (71.0%)
Beta blockers	18 (58.0%)
ACE inhibitors/ARBs	16 (51.6%)
Diuretics	13 (41.9%)
Statins	11 (35.5%)
Calcium channel blockers	7 (22.6%)
Antiplatelet agents	5 (16.1%)
Antiemetics	3 (9.7%)
Alpha blockers	2 (6.5%)
Antiarrhythmic drugs	1 (3.2%)
Antifungal agents	1 (3.2%)
Outcomes	
Mortality	1 (3.2%)
Use of mechanical ventilation	20 (64.5%)
Duration of mechanical ventilation, days	17.0 (IQR 5.5; 18.0), $N=20$ (64.5%)
Use of vasopressors / inotropes	6 (19.4%)
Duration of use of vasopressors / inotropes, days	3.5 (IQR 2.0; 12.0), $N=6$ (19.4%)
Duration of enteral nutrition, days	18 (IQR 17; 20), $N=31$ (100%)
MOF syndrome	8 (25.8%)
Sepsis	7 (22.6%)
Arrythmia	2 (6.5%)
Acute kidney injury	2 (6.5%)
Renal replacement therapy	2 (6.5%)
Bleeding	1 (3.2%)
Respiratory failure	1 (3.2%)
DVT	1 (3.2%)
Nonfatal cardiac arrest	1 (3.2%)
Seizures	1 (3.2%)
Stroke	0
MI	0

Note. ACE — angiotensin-converting enzyme; ARB — angiotensin II receptor blocker; BMI — body mass index; ICU — intensive care unit; IQR — interquartile range; MOF- multiple organ failure; DVT — deep vein thrombosis; MI — myocardial infarction; MV — mechanic ventilation; NYHA — New York Heart Association.

Table 2. Dynamics of clinical, nutritional indicators, and biomarkers of the gastrointestinal tract.

Parameters	Parameter values		*P**
	Day 0, *N*=31	Day 20, *N*=29	
PEM degree			
GLIM	No: 22 (71.0%) Moderate: 6 (19.4%) Severe: 3 (9.7%)	No: 21 (72.4%) Moderate: 5 (17.4%) Severe: 3 (10.3%)	0.9
Russian PEM Scale	Mild: 12 (38.7%) Moderate: 16 (51.6%) Severe: 3 (9.7%)	Mild: 6 (21.4%) Moderate: 21 (75.0%) Severe: 1 (3.6%)	0.8
PNI	37.8 (30.6; 42.0)	36.0 (28.2; 40.4)	0.4
Clinical and anthropometric parameters			
Heart rate (beats/min)	83 (75; 94)	80 (74; 90)	
Body temperature (°C)	36.6 (36.5; 36.8)	36.6 (36.5; 36.8)	0.6
Height (cm)	171 (170; 178)	171 (170; 176)	0.9
Body weight (kg)	75.0 (67.5; 87.0)	75.0 (63.5; 80.0)	0.3
BMI, kg/m²	26.0 (21.6; 29.4)	25.1 (21.3; 27.5)	0.3
Mid-upper arm circumference (cm)	27.5 (25.4; 29.3)	26.5 (23.7; 28.6)	0.07
Triceps skinfold thickness (mm)	9.0 (7.0; 12.0)	10.0 (8.0; 11.5)	0.7
Upper arm muscle circumference (cm)	24.5 (22.6; 25.6)	23.2 (20.3; 24.6)	0.004
Scale scores			
Modified Rankine scale	5 (5; 5)	5 (5; 5)	0.9
The Bartel Index	0 (0; 0) from 0 to 20	0 (0; 0) from 0 to75	0.3
FIM	19 (19; 19)	19 (19; 19)	0.1
Nitrogen excretion rate and gastrointestinal biomarkers			
Nitrogen excretion (g/day)	*N*=27, 8.8 (4.6; 12.3)	*N*=27, 8.7 (3.9; 12.8)	0.4
Fecal zonulin (ng/ml) reference: 0–83.15	*N*=23, 7.5 (4.5; 13.3)	*N*=23, 11.5 (6.5; 36.2)	0.028
Fecal α-1-antitrypsin (mg/L) reference: < 250 (neg.)	*N*=23, 17.3 (8.6; 80.9)	*N*=23, 25.1 (15.1; 74.4)	0.9

Note. PEM — protein-energy malnutrition; GLIM — Global Leadership Initiative on Malnutrition; PNI — prognostic nutritional index; BMI — body mass index; FIM — Functional Independence Measure; ICU — intensive care unit. * — McNemar test, Wilcoxon paired rank test.

dition, patients with PEM had lower mid-upper arm circumference and upper arm muscle circumference at the follow-up (Supplementary Table A4).

There were also no significant differences in clinical scale scores, biomarkers of gut excretion, or hospital outcomes. However, the presence of PEM was associated with significant changes in the composition of the colonic microbiota by Day 20. In particular, patients with PEM had lower *Escherichia coli* titers (P=0.026) and higher *Enterobacter* spp. titers (P=0.020).

By Day 20, patients with moderate and severe protein-energy malnutrition had higher motor activity in the duodenum and jejunum relative to overall motor activity (P (i) / PS (%) for the duodenum — P=0.003, for the jejunum — P=0.006), than patients without PEM. An increase in the P (i) / P (i+1) ratio for the jejunum and ileum (P=0.019) and a decrease in the P (i)/P (i+1) ratio for the stomach and duodenum (P=0.011) were also documented (Supplementary Table A4, Fig. 3).

By means of correlation analysis, several statistically significant relationships between PNI, gut microbiota composition and EGEG parameters were revealed. On Day 0, a moderate inverse correlation between PNI and *Klebsiella pneumoniae* titer (Spearman coefficient R=–0.50, P<0.001) was established, as well as PNI and *Acinetobacter* spp. titer (R=–0.40, P=0.001). Additionally, a moderate inverse correlation was established between individual EGEG parameters and the titer of several microorganisms (additional Figures A1–A2).

Discussion

Moderate or severe nutritional status disorders were detected in approximately one-third of patients with severe TBI/ischemic stroke. During the follow-up, marked deviations in the gut microbiota composition and EGEG parameters from reference microbiome profile were defined, while biochemical markers of intestinal permeability (zonulin and alpha-1-antitrypsin) remained within the normal range. It can be reasoned that PEM was associated with significant dyscoordination in GIT function and substantial changes in gut microbiota composition (decreased *E. coli* titer, P=0.026; increased *Enterobacter* spp., P=0.020).

The results of previous studies indicate that the incidence of PEM in patients with severe brain damage ranges from 6.1% to 62% [26]. PEM etiology is influenced by a variety of factors, but its underlying causes remain poorly understood [27]. It has been established that protein metabolism is the most vulnerable component of nutritional status in patients in chronic critical conditions [9]. Some reports indicate that the fact of brain damage is a sta-

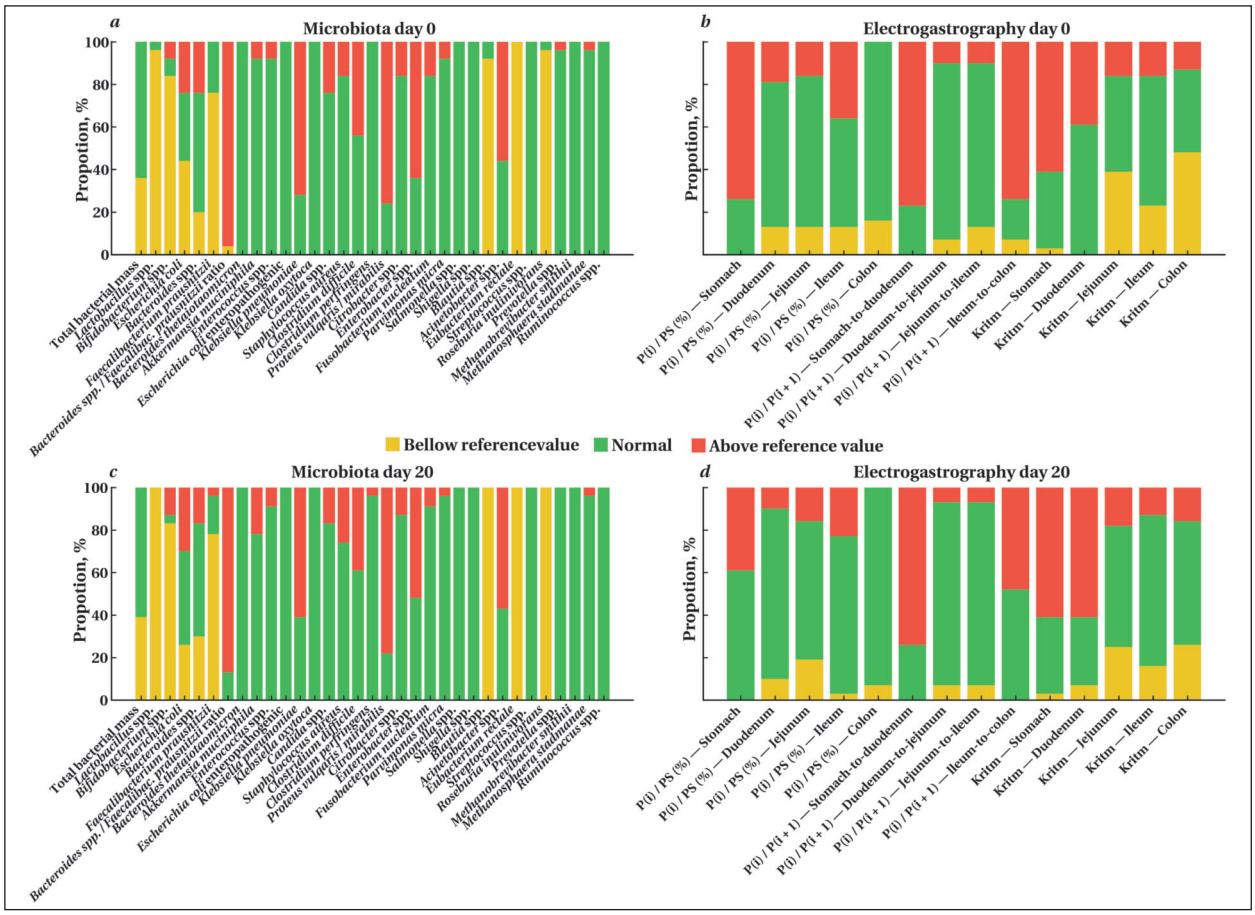

Fig. 2. Composition of gut microbiota determined by qPCR and parameters of electrogastroenterography at baseline — Day 0 (*a, b*), and on Day 20 (*c, d*), presented in comparison with reference values.

tistically significant factor of increased risk of sarcopenia and malnutrition [28, 29]. An increase of small intestine permeability persisting for up to 28 days was reported in mice with modelled TBI, in contrast to controls [30]. In this study, no increase in intestinal permeability was found among examined patients: the concentrations of fecal zonulin and alpha-1-antitrypsin remained within the reference values. There were some reports indicating an increase in zonulin concentration in patients in critical conditions developing sepsis [31, 32]. However, extreme reduction of zonulin activity may signify an attempt to compensate for the loss of intestinal barrier integrity, as shown, for example, in children in the postoperative period [33].

Abnormal chyme transit could be another cause of malnutrition. We suggest that PEM in patients in chronic critical condition may also be associated with uncoordinated gastrointestinal motility. The gut microbiota plays the key role in maintaining homeostasis [34], and severe brain damage may critically affect this principal function leading to inevitable complications.

Intestinal dysbiosis can be a consequence of poor nutrition in the ICU. In particular, K. Shimizu et al. demonstrated a significant decrease in the total titer of obligate anaerobes, including *Bacteroidaceae* and *Bifidobacterium*, and increase in the titer of staphylococci in patients having challenges with enteral nutrition [35]. Eventually this study demonstrated improvement in GIT function, reducing diarrhea episodes after replacement of enteral mixture with natural food through a gastrostomy. Logical to suggest, that this improvement could be associated with modification in gut microbiota composition [36].

A PCR-based testing was used in this study for rapid assessment of the amount of clinically relevant microorganisms in gut microbiota. The results obtained are consistent with findings in patients with severe TBI [37–39], including an increase in the titer of *Enterobacteraceae* and a decrease in saprophytes. Patients with PEM had a lower titer of *Escherichia coli* and a higher titer of *Enterobacter* spp. A decrease in the titer of *E. coli* (a typical representative of healthy microbiota) and an increase in the titer of opportunistic *Enterobacter* spp. indicate an imbalance in the microbiota, supporting the hypothesis that PEM is associated with severe dysbiosis. *E. coli* plays an important role in synthesis of vitamins (K, B12) and fermentation of fiber [40]. A deficiency of these bacteria can exacerbate mal-

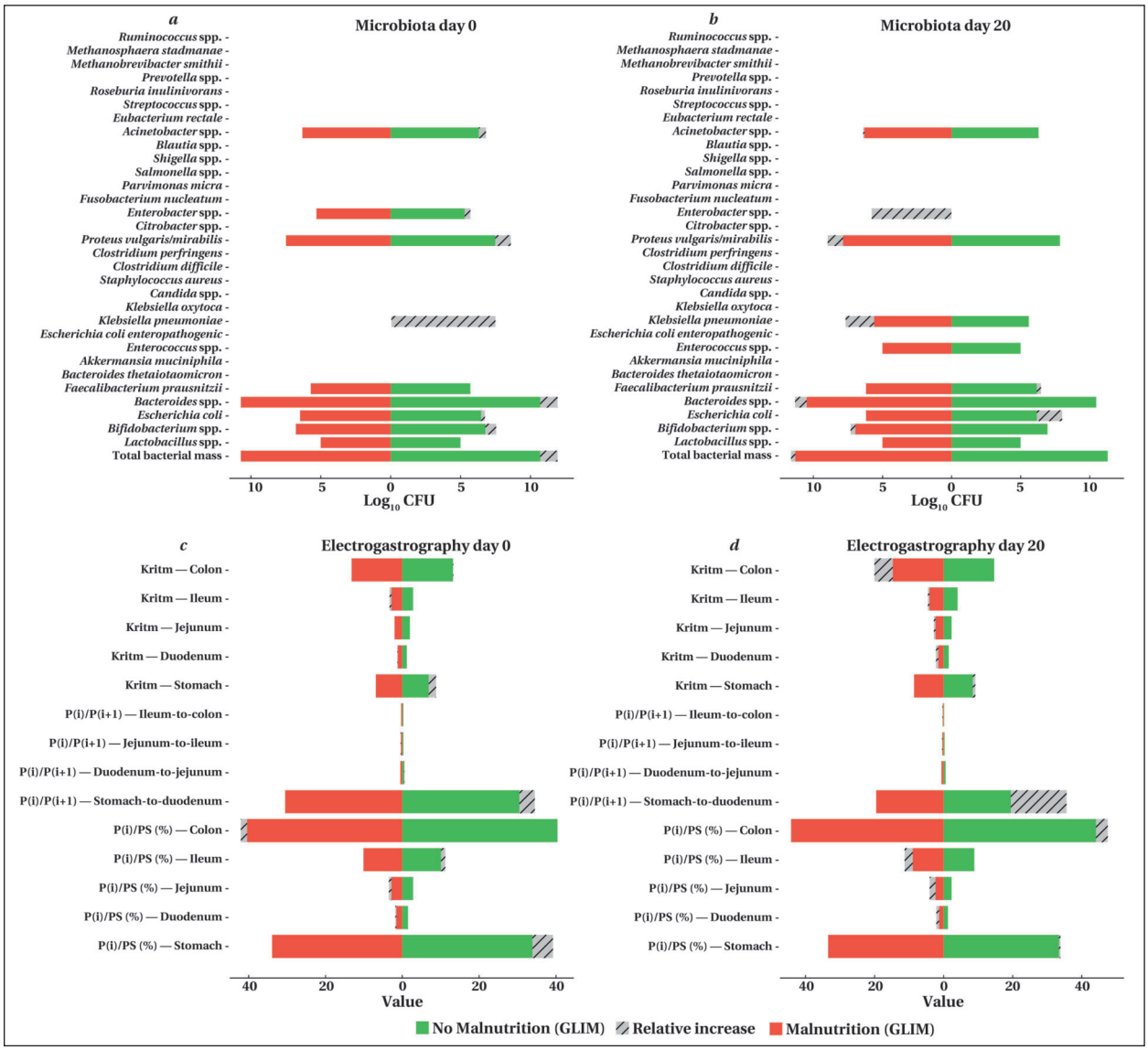

Fig. 3. Comparative analysis of gut microbiota composition (q PCR) and the parameters of electrogastroenterography at baseline, Day 0 (*a, c*) and on Day 20 (*b, d*) in patients stratified by nutritional status according to the GLIM criteria. Median values are presented.

absorption disorders, forming a vicious circle in PEM. This necessitates personalized treatment, including correction of microbiota (probiotics, prebiotics) and simultaneous nutritional support to restore metabolic and immune processes.

Of particular interest are the identified correlations between the titers of individual microorganisms and EGEG parameters. Modified GIT transit time — through the entire length and through individual segments, has a major impact on gut microbiota composition [41]. Reciprocally, the gut microbiota acts co-dependently and regulates microbial colonization of the gut ecosystem by influencing GIT motility [42]. We have identified not only a decrease in peristaltic activity, but also a significant imbalance in intestinal motility, which can also disrupt the growth of the intestinal microflora. However, the detailed mechanisms of this complex regulatory system remain insufficiently studied, especially in critically ill patients, which requires additional research.

We have identified a statistically significant association between PEM development and gut microbiota dysbiosis in combination with GIT motor dysfunction in patients in a chronic critical condition after traumatic brain injury and stroke. A comprehensive therapy in accordance with the approved treatment protocols aimed at improving patients' nutritional status did not bring statistically significant improvement of variables characterizing gut microbiota and intestinal motility. Proven high prognostic significance of PEM in development of adverse clinical outcomes [5] stimulates the search for and implementation of innovative technologies for correcting intestinal dysfunction in clinical practice.

Study limitations. As the sample size was not calculated, it may limit the statistical power to detect clinically significant differences. The single-center observational design does not allow for the generalization of the results to other patient populations and clinical conditions, nor does it establish the nature of causal relationship between GIT dysfunction and development of PEM. Additionally, the incomplete assessment of metabolic processes and gut microbiota composition due to the lack of metagenomic sequencing should be considered. Addressing this methodological gap and conducting multicenter clinical trials will complement the interpretation of results and expand our understanding of the processes been studied.

Conclusion

Development of malnutrition in patients with severe brain damage is associated with both severe gut microbiota dysbiosis and impaired gastrointestinal motility. Specific identifiable changes in gut microbiota included a decrease in *E. coli* and other beneficial microorganisms titers (*Lactobacillus, Bifidobacterium*), an increase in the titer of opportunistic pathogens (*Enterobacter* spp., *Klebsiella*, *Proteus, Acinetobacter*), as well as untoward shifts in the ratio of *Bacteroides* spp. / *Faecalibacterium prausnitzi*. These results emphasize the relevance of considering the state of gut microbiota and GIT motor function when providing nutritional support in intensive care.

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Effect of Rapamycin on Staurosporine-Induced Cardiac Myofibroblast Death

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Summary

Many cardiac diseases are associated with an excessive accumulation of myofibroblasts, characterized by increased production of extracellular matrix proteins and resistance to apoptosis, which leads to progression of fibrosis and cardiac dysfunction. Targeting the mechanisms of myofibroblast elimination is a promising strategy for treating fibrosis that requires further investigation.

The aim of this work was to determine the ability of the autophagy activator rapamycin to affect the staurosporin-induced death of cardiac myofibroblasts.

Materials and methods. *In vivo* modeling of cardiac fibrosis was performed using a mouse model of aortic arch ligation. *In vitro* studies used myofibroblasts obtained by differentiation of cardiac fibroblasts in presence of transforming growth factor beta 1 (TGFb1). To study the mechanism of myofibroblasts elimination, a cell model was developed using staurosporine, an alkaloid that can initiate apoptosis in a culture of cardiac myofibroblasts. The activity of apoptosis and autophagy was studied using immunofluorescence staining, immunoblotting, and flow cytometry.

Results. It was shown that pressure-induced cardiac overload causes the accumulation of myofibroblasts characterized by a low rate of apoptosis (annexin V+ cells in sham-operated hearts and after modeling pressure overload (0.0016 ± 0.0006% and 0.0019 ± 0.0009%; $P = 0.32$, $N = 10$), leading to marked interstitial fibrosis in the myocardium. It was found that rapamycin is able to enhance the effect of staurosporin and cause increased myofibroblast death due to autophagy-associated mechanisms (control 1.68 ± 0.66% ($N = 4$); staurosporin 65.8 ± 2.63% ($N = 4$); rapamycin + staurosporin 73.73 ± 0.67% ($n = 4$); control vs staurosporin $P < 0.0001$; control vs rapamycin + staurosporin $P < 0.0001$; staurosporin vs rapamycin + staurosporin $P = 0.0071$).

Conclusion. Rapamycin enhanced myofibroblast apoptosis induced by staurosporine, which may be related to regulation of the mTOR signaling and increased autophagy activity. The molecular mechanisms of this process require further research.

Keywords: cardiac myofibroblasts; rapamycin; staurosporine; cardiac fibrosis; autophagy

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

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Introduction

Cardiac fibrosis is an important component in the pathogenesis of most cardiovascular diseases, including myocardial infarction, congestive heart failure, cardiomyopathies, arterial hypertension, still remaining an unresolved health problem in advanced economies around the world [1, 2]. Fibrosis is characterized by excessive deposition of extracellular matrix proteins in the interstitium, leading to significant remodeling of the cardiac microenvironment, increased cardiac wall stiffness, and irreversible impairment of cardiac systolic function. In addition, disruption of the myocardial native structure creates an ideal «breeding ground» for ventricular arrhythmias with highest risk of sudden death, and accelerates the progression of heart disease [3–5]. The results of numerous scientific studies provide sufficient evidence to consider fibroblasts/myofibroblasts as the main pathological determinant in development of diseases associated with fibrosis [6]. It is well known that fibroblasts are the most important regulators of the cardiac microenvironment, ensuring the regulation of collagen synthesis/degradation, implementation of

electromechanical coupling, effectuation of a rapid response to damage (e. g., strengthening the infarcted heart wall to prevent its rupture), and exchange of secretory signals [7–9]. Meanwhile, disruption of fibroblasts elimination mechanisms, which are implemented through apoptosis, triggers a «vicious circle» of fibroblast activation, leading to their differentiation into myofibroblasts, their excessive accumulation in cardiac tissue, excessive matrix deposition, and the development of pathology [10]. This determines the high relevance of studies aimed at investigating the molecular mechanisms of myofibroblast pool regulation, and searching for compounds with the potential to induce myofibroblast elimination, as the basis of a strategy preventing progression of fibrosis. In this regard, compounds that modulate autophagy, an evolutionarily conservative mechanism for maintaining cellular homeostasis through utilization of macromolecules and organelles via the lysosomal degradation pathway, are of particular interest [11–12]. Recent studies by our and other research groups have shown, that numerous conditions associated with fibrosis are characterized by decreased autophagy, both in individual subpopulations of fibroblasts/myofibroblasts and in other cells of the cardiac microenvironment [12, 13]. Consequently, increasing autophagy activity within physiological limits may be a potential strategy for influencing myofibroblast function and the mechanisms of fibrosis development in general.

The aim of this study was to determine the potential of the autophagy activator rapamycin to affect staurosporine-induced death of cardiac myofibroblasts.

Materials and Methods

Animals. All animal procedures were approved by the Ethics Committee of the Institute of experimental cardiology of Academician E. I. Chazov National medical research center for cardiology (permit No. LA/28.07.2023 dated July 28, 2023; No. LA/26.09.2023 dated September 28, 2023), and were carried out in accordance with current international standards. The studies were conducted on male C57BL/6J mice weighing 20–23 g. Throughout all experiments, the mice were kept at a temperature of 24°C with a 12-hour day-night cycle and free access to food and water. The animals were euthanized by anesthesia with isoflurane followed by dislocation of the cervical spine.

Modeling of transverse aortic stenosis. The animals were divided into two groups: 1) sham-operated animals (N= 10); 2) animals with modeling of transverse aortic constriction (TAC) (N= 72). Tribromoethanol (avertin, intraperitoneally; 100 µl/kg) was used to anesthetize the animals. The TAC was modeled via access to thoracic cavity provided by midline sternotomy. To facilitate exposure of the aortic arch, the thymus and neck muscles were displaced in the surgical field, and a 6.0 suture thread was inserted under the aorta between the origins of the brachiocephalic and left common carotid arteries. Next, a 27G curved needle was placed on the aortic arch, over which a ligature was tied. After tightening the ligature (around the needle and aorta), the needle was quickly removed. Control mice were subjected to a simialr procedure, but without aortic arch ligation (sham-operated animals). After the procedure, the conventional layered wound closure was employed. Oral panadol syrup was used for postoperative pain relief (for 5 days). The animals were euthanized 30 days after the procedure.

Analysis of cardiac tissue cryosections after TAC modeling. For histological studies, the hearts of the mice were removed, washed with physiological solution, embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek), frozen in liquid nitrogen vapor, and used for cryosection preparation. Cryosections 7 µm thick were prepared on a Leica CM1900 cryostat, placed on slides, and stored at –70°C.

Heart sections were stained with picrosirius red to visualize collagen in accordance with previously described protocols [14–16]. The sections were washed with distilled water and placed in a hot dye solution for 10 minutes. The sections were then washed three times with a 5% acetic acid solution. After staining, the slides were washed with distilled water, dehydrated, and mounted using a xylene-based medium. The stained preparations were examined using standard light and polarized microscopy, and the images were documented using a Leica Aperio CS2 device (Leica). Morphometric analysis was performed by measuring the size of cardiomyocytes using Image J software (NIH, USA).

Assessment of autophagy-regulating proteins expression by immunoblotting. To analyze autophagy activity, samples of left ventricular cardiac tissue from control and TAC-operated animals were used. The lysate proteins were separated by SDS-electrophoresis in a 10% polyacrylamide gel on a Mini- PROTEAN 2 device (Bio-rad, USA). Electrophoresis was performed on a PVDF membrane (Millipore, USA) using a Transblot Turbo device (Bio-rad, USA). After electrotransfer, the membrane was incubated in a blocking buffer (phosphate-saline buffer containing 5% skim milk powder (AppliChem, USA). The membranes were then incubated with antibodies against LC3 II/I (Abclonal, USA), p62/SQSTM1 (Abclonal, USA), the active form of caspase 3 (cleaved caspase-3; Cell Signaling, USA) and tubulin (Cell Signaling, USA) for 12 hours at +4°C with constant stirring. The membranes were then washed three times in PBS containing 0.05% Tween-20 (each wash for 10 minutes with constant stirring). After washing, the membranes were incubated with secondary antibodies against rabbit immunoglobulins conjugated with horseradish peroxidase AffiniPure (H + L) (Jackson ImmunoResearch, USA). The mem-

branes were then washed three times for 10 minutes each in phosphate-buffered saline containing 0.05% Tween-20. Protein detection was performed using SuperSignal West Pico chemiluminescent substrate (Thermo Scientific, USA). The signal was recorded using the Fusion-SL 3500.WL gel documentation system (Vilber Lourmat, France). Densitometric analysis of luminescence intensity of the studied proteins was performed using Image J software (National Institutes of Health, USA).

Obtaining a culture of mouse cardiac myofibroblasts. Mouse cardiac tissue (intact hearts of C57BL/6J mice; *N*=160) was minced and fermented in a solution of 0.1% type II collagenase and 0.1% trypsin (10 times for 10 minutes at 37°C). The isolated cells were resuspended in modified DMEM/F12 medium containing 10% fetal calf serum and antibiotics. After 120 minutes, the floating cells were washed with PBS, and the adhered cells were used for culture expansion for 3 days. Next, the obtained cells were deprived and stimulated with recombinant TGFb1 (5 ng/ml) for 4 days to induce fibroblast differentiation towards myofibroblasts for obtaining a myofibroblast culture [17–19].

Detection of autophagosomes using Cyto-ID dye. Cyto-ID is a dye that selectively detects autophagosomes with minimal staining of lysosomes, allowing it to be used for specialized monitoring of autophagy [20]. The Cyto-ID® Green Detection Kit (Sigma, USA) was used to visualize autophagy vesicles. Mouse cardiac myofibroblasts (1×10^4) were cultured on glass slide chambers in the absence or presence of staurosporine. The sample was stained with Cyto-ID® reagent for 30 minutes at 37°C and analyzed using Image Exfluorer (LCI, South Korea).

Evaluation of myofibroblast characteristics. Immunocytochemistry was used to detect specific myofibroblast markers (collagen, smooth muscle alpha-actin). Cells were cultured in sterile culture plates with wells, washed, and fixed for 10 minutes in a 3.7% formaldehyde solution prepared in phosphate-saline buffer (pH 7.3). Next, the cells were washed three times with PBS, permeabilized in a 0.1% solution of Triton X100/PBS (10 minutes), and after washing with PBS, the cells were incubated in a «blocking» solution that prevents nonspecific binding of II antibodies (1% BSA/PBS solution, 10% serum from the animal donor of II antibodies). The slides were then stained with primary and II antibodies conjugated with Alexa Fluor 594 (Invitrogen, USA). Cell nuclei were stained with DAPI (4',6-diamidino-2-phenylindole). Phalloidin-Alexa594 (a bicyclic oligopeptide capable of selectively binding to polymerized fibrillar actin, a component of the cytoskeleton) was used to evaluate the cytoskeleton.

The «collagen gel contraction» assay was used to assess the contractile phenotype of myofibroblasts. Collagen I (2.5 mg/ml) was used as the carrier gel. The collagen gel was obtained by diluting Viscol (Imtek, Russia) with 0.1 N NaOH solutions and 10× culture medium. Cell suspension (fibroblasts and myofibroblasts) was mixed with ready-made collagen gel (at a ratio of 2×10^5cells per 1 ml of carrier gel) and seeded at 250 µl per well in a 48-well plate (Corning, USA). After gel polymerization, 0.5 ml of medium was added to each well and cultured at 37°C, 5% CO_2. After 72 hours, the change in gel area reflecting the contractile ability of the cells was recorded.

Assessment of myofibroblast apoptosis rate. The rate of myofibroblast apoptosis in heart sections of sham-operated and TAC mice was studied using the TUNEL in situ cell apoptosis kit in accordance with the manufacturer's recommendations (Rosch, USA). To assess myofibroblast apoptosis after exposure to staurosporine (50 nM) and staurosporine in combination with rapamycin (10 µM) *in vitro*, flow cytometry was used in combination with double staining with annexin V and propidium iodide in accordance with the kit manufacturer's instructions (BD Pharmingen, USA). Attached cells were collected with trypsin-EDTA solution, combined with detached cells, washed in PBS, and resuspended in binding buffer. Next, annexin V and propidium iodide were added, and the mixture was incubated at room temperature for 30 minutes before analysis. The analysis was performed by flow cytometry using BD FACS Aria III (BD Pharmingen, USA). The number of annexin V+ apoptotic cells was calculated as a percentage of the total population.

Microscopy and image analysis. Cells and cryosections of the myocardium were analyzed using an Axiovert 200 M fluorescence microscope (Carl Zeiss, USA) and AxioVision 4.8 software (Carl Zeiss, USA).

Statistical analysis. Data are presented as mean ± standard deviation (*M* ± *SD*). Statistical analysis of data was performed using GraphPad Prism software (GraphPad Software 8.3.0). Normality of distribution was tested using the Shapiro–Wilk test. Statistical comparison of *in vivo* study data was performed using Student's *t*-test. Comparison of in vitro study data was performed using one-way ANOVA with Welch's correction, and intergroup pairwise comparisons were performed using Holm–Sedak multiple comparison test. Differences were considered statistically significant at $P<0.05$.

Results

To assess the activity of apoptosis in cardiac fibroblasts, we used *in vivo* modeling of cardiac fibrosis caused by pressure-induced cardiac overload. Animals survival rate on the 30th day of follow up after TAC procedure was 14%. There were no late infection complications after TAC surgery. Signs of LV hypertrophy and marked interstitial and perivascular fibrosis, detected by picrosirius red stain, were documented in 30 days after TAC procedure (Fig. 1, *a, b*). Assessment of

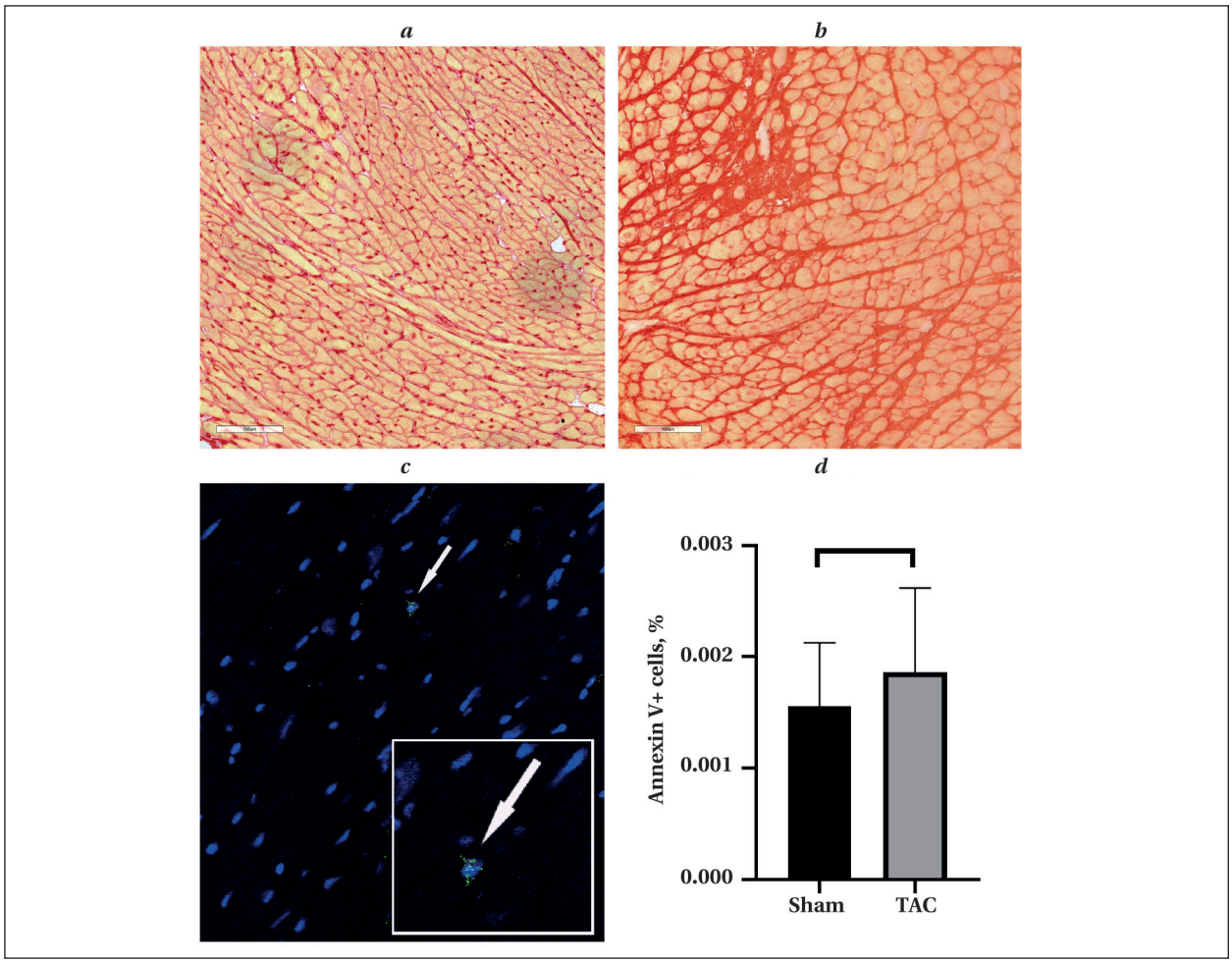

Fig. 1. The development of cardiac fibrosis was characterized by low rates of fibroblast/myofibroblast apoptosis.
Note. Representative images of heart sections from sham-operated mice *(a)* and animals after TAC *(b)*. Stained with picrosirius red. Collagen fibers are outlined in red. Representative images of annexin V+ cell detection (green in the square field), reflecting their entry into apoptosis, in the heart of a mouse after TAC modeling *(c)*. Cell nuclei are stained with DAPI. The arrow points to an annexin V+ cell. *d* — the bars represent quantitative assessment of annexin V+ cell content in sham-operated and TAC hearts. Statistical analysis was performed using Student's *t*-test; *staurosporine*=0.32, *N*=10).

apoptosis rate using the Tunel assay showed that only a few cells in the interstitium had positive staining, indicating the initiation of cell death mechanisms (Fig. 1, *c*). Meanwhile, the total number of Tunel+ cells in cardiac interstitium of animals in the sham-operated and TAC groups did not differ (Fig. 1, *d*). Therefore, fibrogenesis in the heart was characterized by low rates of fibroblast/myofibroblast death.

To clarify the mechanisms of myofibroblast resistance to apoptosis, a myofibroblast culture was obtained by exposing primary cardiac fibroblasts to the profibrotic cytokine TGFb1. TGF-β treatment led to changes in the morphology of cardiac fibroblasts: spindle-shaped cells increased in size, and a network of stress fibers formed based on polymerized actin microfilaments (Fig. 2, *a, b*). The stress fibrils included smooth muscle α-actin fibers, indicating cytoskeletal reorganization and acquisition of a contractile phenotype by the cells (Fig. 2, *b*). Indeed, treatment of cells with the profibrotic inducer TGFb1 led to the generation of cytoskeletal tensile forces that caused contraction of the collagen-formed gel (Fig. 2, *c, d*). Thus, treatment of cells with TGFb1 led to a change in cell morphology, reorganization of the cytoskeleton with the formation of a branched network of stress fibrils, increased expression of smooth muscle alpha-actin, collagen 1, and the acquisition of contractile ability, indicating their differentiation towards myofibroblasts. (Fig. 2, *b*).

To study the mechanism of myofibroblast elimination, an *in vitro* cell model was developed based on cell culture in the presence of staurosporine. Rounded cells with signs of chromatin condensation appeared just 6 hours after treating myofibroblasts with staurosporine (Fig. 3). To confirm the induction of cell death after the addition of staurosporine, we performed immunoblotting studies and found the formation of cleaved caspase 3, indicating the activation of the intrinsic apoptosis signaling pathway (Fig. 3, *d*). In addition, we found that the above

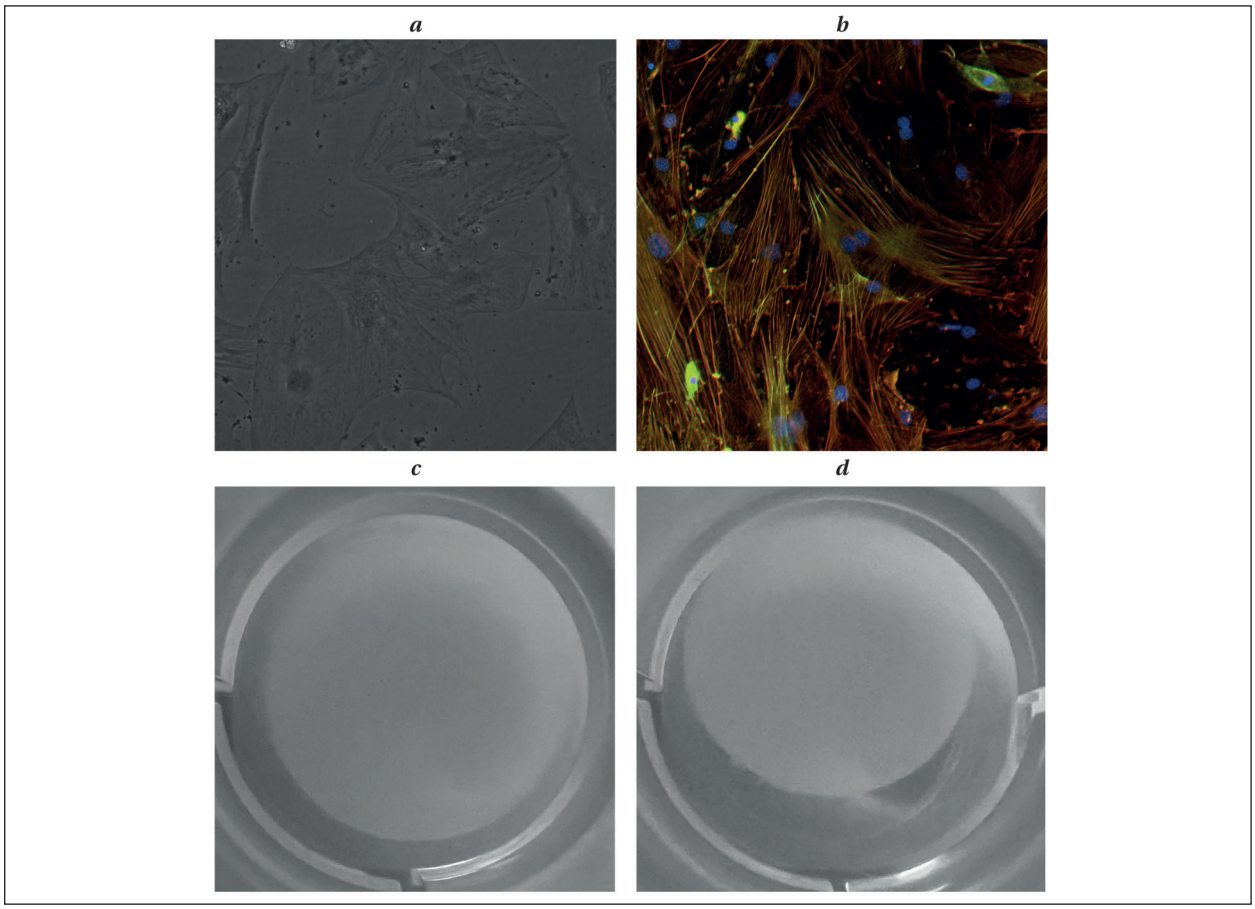

Fig. 2. Characteristics of mouse heart myofibroblasts.
Note. *a* — representative images of mouse heart myofibroblasts (phase contrast). *b* — representative images of immunofluorescence staining of myofibroblasts with antibodies to smooth muscle alpha-actin (green) and phalloidin (red); Yellow indicates co-localization of the smooth muscle alpha-actin and phalloidin signals. *c, d* — representative images of collagen gel contraction containing fibroblasts *(c)* and myofibroblasts *(d)*.

manifestations were accompanied by the accumulation of autophagosomes, as shown by the CytoID assay using specific dye, and signs of autophagy machinery activation (increased LC3II/LC3I ratio and p62/SQSTM1 degradation) (Fig. 3, *c–e*). One day after staurosporine treatment, CytoID+ vacuoles accumulated in most apoptotic cells, indicating a link between cell death and autophagy.

Next, we decided to test how addition of rapamycin, which causes an increase in autophagy activity, could influence the rate of apoptosis in myofibroblasts induced by staurosporine. We found that culturing cells in the presence of rapamycin had no effect on the viability of myofibroblasts. However, its combined use with staurosporine enhanced cell death, emphasizing the important role of increased autophagy activity as a trigger for apoptosis (control 1.68 ± 0.66% (N=4); staurosporine 65.8 ± 2.63% (N=4); rapamycin+staurosporine 73.73 ± 0.67% (N=4); control vs staurosporine $P < 0.0001$; control vs rapamycin+staurosporine $P < 0.0001$; staurosporine vs rapamycin+staurosporine p=0.0071) (Fig. 3, *e*).

Discussion

A sound evidence has been accumulated for considering myofibroblasts as key cells responsible for excessive synthesis, deposition, and remodeling of extracellular matrix proteins in cardiac fibrosis [7–10]. Despite certain success in identifying biological inducers and understanding the background of fibroblast differentiation towards myofibroblasts, the exact mechanisms that support myofibroblasts persistence in fibrotic tissues remain poorly understood.

In this study, we showed that pressure-induced cardiac overload causes accumulation of myofibroblasts population with a low rate of caspase-dependent cell death, and development of noticeable perivascular and interstitial fibrosis in the myocardium. An *in vitro* cell model was developed to study the mechanism of myofibroblast elimination. It is based on the use of staurosporine, an alkaloid that can initiate apoptosis in cardiac myofibroblast cultures through caspase-dependent and caspase-independent mechanisms [21–23]. We found that rapamycin can enhance the effect of staurosporine

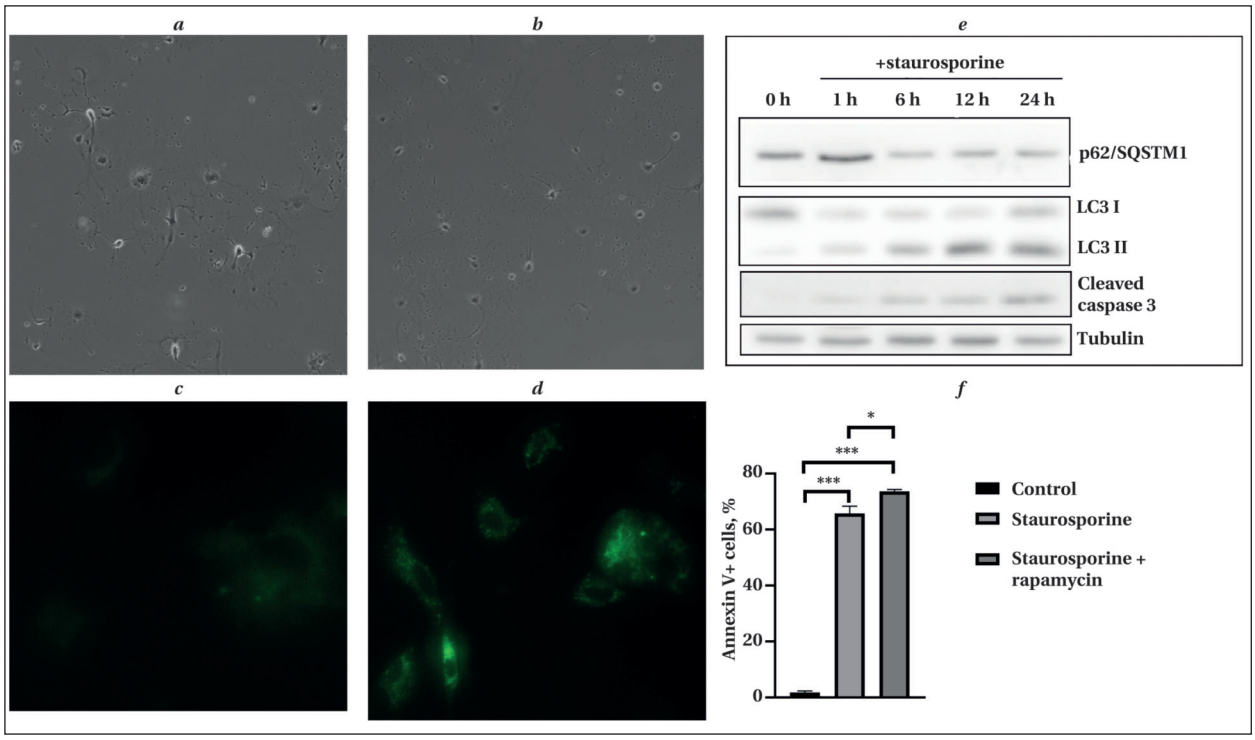

Fig. 3. Staurosporine activated autophagy and caused myofibroblast death.
Note. *a, b* — representative images of myofibroblasts (phase contrast); *a* — after treatment with staurosporine (50 nM, 24 hours); *b* — after treatment with staurosporine (50 nM, 24 hours) in combination with rapamycin (10 μM, 24 hours). *c, d* — representative images of Cyto-ID+autophagosomes (green); *c* — in control myofibroblasts; *d* — after treatment with staurosporine. *e* — representative images of membranes stained with antibodies to p62/SQSTM1, LC3 I, II, cleaved caspase 3, and tubulin, reflecting protein expression in control myofibroblasts and those treated with staurosporine (50 nM, 1, 6, 12, and 24 hours). *f* — graphs showing quantitative assessment of apoptosis rates in control myofibroblast culture, cells after treatment with staurosporine, and treatment with staurosporine in combination with rapamycin for 24 hours. Statistical analysis was performed using ANOVA with Welch's correction (*** — $P < 0.0001$; * — $P = 0.0071$; $N = 4$ in each group).

and cause increased myofibroblast death through autophagy- associated mechanisms.

Autophagy is an evolutionarily conservative process that participates in maintaining homeostatic balance between the synthesis, degradation, and recycling of organelles and proteins to meet metabolic needs and regulate cellular functions [24–26]. As an adaptive response, autophagy can alleviate stressful conditions such as hypoxia, oxidative stress, starvation, endoplasmic reticulum stress, etc. [27]. In the early stages, it is cytoprotective, but its prolonged activation can lead to cell organelle dysfunction, the initiation of the apoptotic cascade, and cell death (programmed type II cell death) [28, 29]. The serine/threonine protein kinase mTOR (mammalian target of rapamycin) plays the key role among all sensors regulating autophagy [30]. Under conditions of nutrient availability, it can inhibit the autophagosome initiation complex — ULK (Unc-51-like kinase 1 complex (ULC)). The mTOR is also involved in the regulation of autophagy-related gene transcription. It can phosphorylate the TFEB transcription factor, preventing its nuclear translocation and transcription of a number of genes associated with autophagy [31–35]. Studies conducted by our group have shown that the development of cardiac fibrosis caused by pressure-induced cardiac overload is characterized by decrease of autophagy activity in cardiac tissue. Detailed bioinformatic studies of single cell transcriptome revealed no change in the expression of genes associated with autophagy in the entire pool of cardiac fibroblasts and its individual subpopulations during the development of cardiac dysfunction [12]. This can be explained by preservation of high mTOR activity, which is responsible for reducing intracellular collagen degradation via autophagy. It mediates cell resistance to apoptosis, and impairs their ability to adapt to stress [36]. To test this hypothesis, we conducted in vitro experiments using the apoptosis inducer staurosporine. This compound was originally obtained from *Streptomyces* and was characterized by its ability to inhibit protein kinase C [37]. However, it was later found to be a non-selective inhibitor of a number of different kinases [38, 39], which gives it the potential to initiate apoptosis in most mammalian cells. In our study, we found that treatment of cells with staurosporine initiated the death of myofibroblasts. It was accompanied by simultaneous increase in the number of autophagosomes and a change in protein expression

(an increase in the LC3II/LC3I ratio and degradation of p62/SQSTM1), associated with increased autophagy activity. A possible explanation for the staurosporine-induced increase in autophagy lies in modified mTOR activity and phosphorylation of downstream translational regulatory components [40]. These include p70 S6 kinase, which phosphorylates ribosomal protein S6 and eukaryotic initiation factor (eIF)4E. Exposure to staurosporine led to a decrease in p70 S6 kinase activity, dephosphorylation of ribosomal protein S6, increased binding of 4E-BP1 to eIF4E, and a simultaneous decrease in the amount of eIF4F complexes. Thus, disruption of mTOR signaling by staurosporine initiates an early cellular response (prior to caspase cascade activation) to a stress stimulus, including that achieved through activation of autophagy, and defines the readiness for the initiation of apoptosis.

Next, we tested how additional stimulation of autophagy can affect the rate of myofibroblasts apoptosis induced by staurosporine. For this purpose, we used rapamycin (sirolimus), an antibiotic/immunosuppressant produced by the soil bacterium *Streptomyces hygroscopicus*, which induces autophagy by acting on its molecular target, mTOR (mammalian target of rapamycin) kinase [24]. In our study, the use of rapamycin significantly enhanced the proapoptotic effect of staurosporine, which may be associated with more powerful and sustained inhibition of mTOR signaling [41, 42].

Conclusion

Thus, pressure-induced cardiac overload causes accumulation of myofibroblasts, characterized by a low apoptosis rate, and the development of marked interstitial fibrosis in the myocardium. To study the mechanism of myofibroblast elimination, a cell model was developed using staurosporine, an alkaloid capable of initiating apoptosis in a culture of cardiac myofibroblasts. It was found that rapamycin can enhance the effect of staurosporine and cause increased myofibroblast death through autophagy-associated mechanisms. The molecular mechanisms of this process require further study.

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Medical Rehabilitation of Children with CNS Tumors Developing State of Minimal Consciousness

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Summary

Objective. To evaluate clinical characteristics and results of individualized medical rehabilitation in children with brain tumors, including those with tracheostomy and/or gastrostomy, upon recovery from a state of minimal consciousness.

Materials and methods. A prospective observational study included 309 patients aged 6 to 17 years who completed treatment for central nervous system (CNS) tumors during 2019–024 yy. at the «Russkoye Pole» medical rehabilitation research center of the Dmitry Rogachev National medical research center for pediatric hematology, oncology, and immunology, Russian Ministry of Health. All patients underwent a comprehensive assessment of treatment effects, including clinical and functional examination, testing of cognitive and emotional functions, and individualized medical rehabilitation. A subgroup of subjects in a state of minimal consciousness with tracheostomy and/or gastrostomy included 9 patients (2.9%). Rehabilitation goals were set based on the domains of the International Classification of Functioning, Disability and Health (ICF), according to an interdisciplinary approach.

Results. Severe neurological deficit as the sequelae of cancer treatment toxicity was documented in 90% of children with CNS tumors. However, all 309 patients achieved the goals of specialized medical programs within 14–21 days. Patients in a state of minimal consciousness most often had impaired breathing, swallowing, eating, communication, and emotional regulation, endangering with life-threatening complications. Adhering to the protocols adapted for management of such patients helped minimize the risk of life-threatening complications. No serious complications were recorded in any patient during the rehabilitation process.

Conclusion. Even in cases of severe functional impairment, including patients requiring tracheostomy and gastrostomy, an individualized medical rehabilitation approach allows for stabilization of patient's condition, upregulation of basic vital functions, and improvement quality of life of patients and their families. Restoring cognitive and sensorimotor functions in children in a state of minimal consciousness requires early initiation of rehabilitation measures by a multidisciplinary team, including a resuscitator, pediatrician, physical therapist, speech therapist, medical psychologist, etc.

Keywords: central nervous system tumors; minimal consciousness; medical rehabilitation; children; tracheostomy; gastrostomy; multidisciplinary approach

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

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Introduction

Recovery from a minimally conscious state manifests by gradual recovery of cognitive functions to a state that is equivalent to chronic impairment of consciousness [1].

Central nervous system (CNS) tumors are the second most common type of malignant neoplasms in children after leukemia, and the most common type of solid tumors, accounting for up to 20% of all cases of cancer in children. Thanks to advances in mainstream medicine, including the use of combined chemo-radiotherapy (CRT), survival rates have improved significantly in recent decades. Currently, about 70–80% of children with CNS tumors achieve five-year survival [2].

Introduction of modern chemo-radiotherapy protocols has led to significant progress in treatment of CNS tumors. However, the malignant growth site and high toxicity of advanced treatments cause long-term disruption in functioning of virtually all organs and systems of the body. At the same time, the need for tracheostomy and gastrostomy most often arises in patients with damage to the brain stem, to cranial nerves, or in patients with severe bulbar dysfunction, requiring tracheostomy in 7–10% of cases [12]. More than 80% of patients with documented cure of CNS tumors are disabled since childhood, as the arising long-term complications and sequelae of therapy during or after treatment, requiring a multidisciplinary approach at the rehabilitation phase remains one of currently unresolved challenges. Restoring consciousness and cognitive functions lost because of the disease and its treatment requires a significant amount of time [3]. It is also emphasized that recovery of cognitive and sensorimotor functions in children in a state of minimal consciousness requires early initiation of rehabilitation measures involving a multidisciplinary team, including a resuscitator, physical therapist, speech therapist, and medical psychologist [11].

Some patients who have undergone neurosurgical interventions for CNS tumors develop swallowing disorders, weakness of the respiratory muscles, and a need for nutritional and respiratory support, which increases the risk of life-threatening complications. In this regard, tracheostomy and gastrostomy are used in this group of children with CNS tumors to provide respiratory and nutritional support.

Tracheostomy and gastrostomy significantly improve life support indicators; however, these procedures are associated with a risk of developing stoma infection, obstruction of the cannula or tube, proliferation of granulation tissue, and septic conditions. This is confirmed by a global survey that revealed insufficient staff training and lack of standardized protocols, which exacerbates the risk of complications [4].

Young children and patients with concomitant neurodegenerative, metabolic, and immunosuppressive conditions are particularly vulnerable in this regard. The lack of standardized protocols for managing patients in a minimally conscious state during the rehabilitation phase, as well as a shortage of trained personnel and difficulties in home care, further increase the risk of adverse events.

A recent review of tracheostomy in children published in Respiratory Care points to increased mortality in this particular patient group, and emphasizes the importance of caregiver training in care and emergency response to reduce the number of emergency hospitalizations [5]. In addition, a 2025 systematic review showed a significant reduction in the quality of life of parents who cared for children with stomas: approximately 40% of mothers experienced moderate or severe stress, and the key risk factors were the young age of the child, comorbidities, and lack of social support [4].

According to recent studies, gastrostomy in children receiving home enteral nutrition is associated with a high risk of complications. Among the most common are infections in the stoma area, excessive growth of granulation tissue, leakage of gastric contents, and displacement or extrusion of the gastrostomy tube. The incidence of these complications reaches 73%, with up to 14% of cases categorized as severe and requiring repeated medical interventions, including surgical correction or device replacement [6].

Specialized inpatient rehabilitation, carried out in accordance with Order No. 878n of the Ministry of Health of the Russian Federation dated October 23, 2019, «On Approval of the Procedure for Organizing Medical Rehabilitation of Children», provides comprehensive support for stoma-treated children who have undergone neurosurgical interventions. The organization of care involves the participation of a multidisciplinary rehabilitation team (MDRT). However, the available literature on medical rehabilitation of children who have undergone CNS tumors is quite limited [14].

Materials and Methods

From 2019 to 2024 a prospective observational study was conducted at the «Russkoe Pole» medical and rehabilitation research center of the Dmitry Rogachev National Medical Research Center for pediatric hematology, oncology, and immunology of the Ministry of Health of Russia (NMRC PGOI named after Dmitry Rogachev, MoH of Russia), focused on the long-term complications and consequences of treatment in children with CNS tumors.

Criteria for inclusion of patients in the study

- CNS tumor in remission or long-term stable disease after specific treatment, including chemo-radiotherapy;

- Age 6 to 18 years;
- Informed consent signed by parents or children over 14 years of age.

Exclusion criteria:

- Severe mental disorders, mental retardation, amblyopia, or other disorders that make it difficult to communicate with the child, infectious diseases;
- Refusal of participation by parents or patient;
- Recurrence of the underlying disease.

The required sample size was not calculated, as the aim was to fully evaluate the center's patient cohort.

The study included 309 patients who were in transition from a state of minimal consciousness. The children's ages ranged from 6 to 17 years, *Me = 11.00 [9.00; 15.00]; min = 6, max = 17.*

There were 254 patients in remission, accounting for 82.2%, and 55 patients, or 17.8%, in a state of long-term stable disease; all children completed therapy for CNS neoplasms. The median follow-up time after discontinuation of therapy was 25.00 months [12.00; 65.00]; min = 1, max = 123.

Boys prevailed in the group, accounting for 179 (57.9%) patients; there were 130 girls (42.1%). This distribution is generally characteristic of the epidemiology of CNS tumors. The median age at tumor onset was 7.5 years [5;10]; min — 0.3, max — 16. The majority of patients — 196, or 63.4% — had a tumor located infratentorially.

The study protocol was reviewed and approved by the Ethics committee of the Dmitry Rogachev National Medical Research Center for pediatric hematology, oncology, and immunology of the Ministry of Health of the Russian Federation (No. 8e/13-17 dated October 27, 2019).

The study complies with STROBE recommendations for cohort studies.

The Glasgow Coma Scale (GCS) and the Coma Recovery Scale — Revised (CRS-R) were used to assess the state of minimal consciousness.

Data were described using measures of central tendency and variability: median and range (min–max) for quantitative variables, and frequency and percentage for categorical variables. All analyses were performed using StataBE 18 (StataCorp, USA).

Results

A methodology for comprehensive assessment of the severity of long-term complications and consequences following the use of various cancer treatment modalities, including different chemotherapeutic agents and dosing regimens, and radiotherapy has been developed. During diagnostic elaboration involving examinations by relevant specialists, laboratory and functional tests, not only the fact and severity of reported clinical manifestations of damage to the nervous, endocrine, cardiovascular, digestive, and other organ systems were established, but also missed, though predictable, long-term consequences were identified, and the severity of monitored complications was clarified.

Screening of neurocognitive functions using a basic test package, including verbal and nonverbal tests, attention span tests, thinking speed, processing, executive functions, as well as components of emotional and behavioral assessment (Cambridge Assessment Neuropsychological Test Battery, Achenbach questionnaire, subtests from the cognitive tests battery (CTB), and Kaufman Assessment Battery for Children) showed a decline in these functions in all 309 children.

A quantitative assessment of the severity of long-term consequences on the CTCAE v 5.0 scale (National Cancer Institute Common Terminology Criteria Adverse Events) in a group of 309 children is presented in the figure.

As demonstrated by the diagrams, in more than 90% of children, the consequences of cancer treatment toxicity were represented by severe neurological deficits; more than 75% of children had endocrine system disorders; 17% of pediatric patients experienced visual complications caused by cancer treatment toxicity, and more than 18% of children had musculoskeletal disorders.

The rehabilitation program lasted 14–21 days and included the use of physical rehabilitation equipment, massage, physiotherapy, as well as individual neuropsychological rehabilitation programs for children and their parents. The package of specialized rehabilitation interventions included correction of postural function, articulation and breathing exercises, speech therapy massage, immediate correction of vision with glasses, medication to correct digestive function, voice and speech correction, gradual transition to independent feeding (as part of nutritional support), training in walking up the stairs and walking independently.

In all 309 cases, the set goals were achieved.

It is evident that due to the severity of organ damage in minimally conscious patients following treatment of CNS tumors, there's a strong background for development of a critical condition during intensive rehabilitation procedures.

Nine (2.9%, 5 girl and 4 boys) out of 309 patients were subjected to tracheostomies (7 cases) and/or gastrostomies (5 cases), including 3 patients requiring both stomas, due to development of a life-threatening condition in the acute or subacute period.

Patients' level of consciousness was assessed upon admission and monitored during hospital stay. The baseline assessment included the Glasgow Coma Scale (GCS) and the Coma Recovery Scale — Revised (CRS-R). Median GCS score on admission was 9 (min 8, max 11), and median CRS-R score was 10 (min 8, max 12), meeting diagnostic criteria for the minimally conscious state (MCS). Therefore,

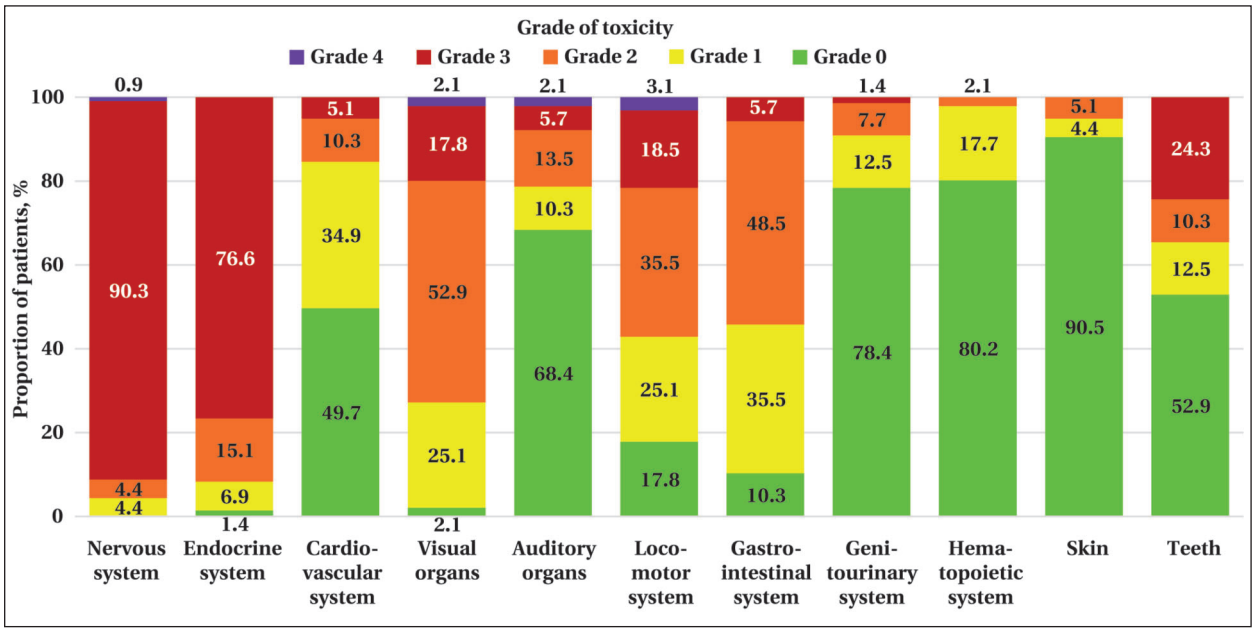

Fig. Toxicity of treatment in patients with CNS tumors according to the NCI-CTCAE v.5.0 scale

all patients in the study group were categorized as having severely altered consciousness and requiring specialized medical rehabilitation measures.

Prior to inclusion into medical rehabilitation program, all patients with tracheostomies had multiple episodes of aspiration in the acute or subacute period that required emergency airway management. Safe enteral feeding was a reliable solution in patients with high risk of aspiration.

Rehabilitation diagnoses, goals, and objectives according to the International Classification of Functioning (ICF) Disability and Health in patients with stomas after treatment of CNS tumors are summarized in Tables 1–3.

Given the severity of illness, challenges with breathing, feeding, and communication, patient's state of minimal consciousness, and vital need for multidisciplinary care, the goals of medical rehabilitation were established using the International Classification of Functioning (ICF) Disability and Health. All patients with gastrostomies and tracheostomies had scores of 4–5 on the Rehabilitation Routing Scale (RRS), which indicated a continuous need for specialized comprehensive rehabilitation. One child had severe sequelae after subtotal removal of a tumor in the posterior cranial fossa and following treatment, including bulbar and ataxic syndromes, bilateral lagophthalmos due to facial nerve palsy, partial optic nerve atrophy (PONA), dysarthria, dysphagia, and dysphonia.

After a 14–21-day course of rehabilitation, the indicators improved: the median GCS increased to 13 scores (min 11, max 14), and the CRS-R increased to 15 scores (min 12, max 17). Two patients improved up to recovery from minimally conscious state.

Improvements in patients' quality of life were achieved through gradual recuperation of the following impaired functions: b770 gait pattern, b320 articulation, b440 breathing, b310 voice and speech, b510 swallowing, b530 weight maintenance, b152 emotional.

Medical rehabilitation in patients with stomas was aimed at minimizing the risks of life-threatening situations. The program envisaged achieving safe, stable movement pattern taking into account the patient's needs, recovery of voice (with the use of alternative or assistive methods of voice and speech communication), recovery or development of oral feeding skills (taking into account the current feeding method), training of communicative (speech-producing) breathing skills, stabilization of patient's emotional state, reduction of anxiety and emotional lability.

Rehabilitation diagnoses, goals, and objectives of medical rehabilitation were formulated for each patient, taking into account the body's structure and functions, as well as the individual characteristics of the patient's activity.

Tables 1–3 present assessments of the progress in terms of achieving the stated goals during hospital stay (at admission and at discharge) in the main rehabilitation domains.

Following specialized medical rehabilitation, including comprehensive respiratory support in

Table 1. Recovery of functions in the study cohort.

ICF domain «body functions» codes and categories	Rehabilitation goals and objectives	Assessment		MDRT participant	IMRP
		O1 upon admission	O2 upon discharge		
b770 Gait pattern functions	Restoration of a safe, stable gait pattern within the ward/home, taking into account the patient's needs	2	1	Physical therapy doctor	Correction of postural function
b320 Articulation functions	Providing background for recovery of articulation motor skills, for nonverbal and speech interaction using available communication channels	3	2	Speech therapist	Articulation exercises
b440 Respiration functions	Optimization of respiratory functions, including patients with tracheostomy; adjustment of breathing allowing speech	3	3	Physical therapy doctor, speech therapist	Breathing exercises
b310 Voice and speech functions	Providing background for recovery or establishment of voice function, using alternative or augmentative means of voice and speech communication	3	2	Speech therapist	Assessment and correction
b510 Swallowing functions	Establishing safe and functional swallowing with minimal risk of aspiration, recovery or development of oral feeding skills	2	1	Speech therapist	Speech therapy massage, correction of dysphagia
b210 Seeing		8	3	PRM doctor, ophthalmologist	Assessment and corrective eyewear/surgery
b515 Digestive functions		2	1	PRM doctor	Pharmacological correction
b530 Weight maintenance functions	Ensuring safe and adequate food intake taking into account the current feeding method and patient's swallowing function	2	1	PRM doctor, nutritionist	Nutritional support, medication adjustment Gradual transition to independent eating
b152 Emotional functions	Stabilization of patient's emotional state, reduction of anxiety and emotional lability	2	1	Psychologist	Correction of emotional state

Note. For Tables 1–3: IMRP — individual medical rehabilitation program; PRM — physical and rehabilitation medicine.

Table 2. Recovery of activities and participation (action) in the study cohort.

ICF codes and categories Domain «activities and participation»	Rehabilitation goals and objectives	Assessment		MDRT participant	IMRP
		O1 upon admission	O2 upon discharge		
d451 Overcoming obstacles (going up and down stairs)	After 7 days, the patient will be able to safely climb and descend 5 flights of stairs independently, without assistance and with minimal fatigue.	22	11	Physical therapy doctor	Staircase walking exercise
d450 Walking long distance	The patient is able walk set distance (e.g., from 100 to 300 meters) independently without outside assistance and with minimal fatigue in the rehabilitation course (usually 14–21 days)	22	11	Physical therapy doctor, PRM doctor	Training to walk independently
d550 Eating	Patient after 14 days can independently and safely take regular consistency food without Assistance, within adequate time	22	11	PRM doctor, dietitian, speech therapist	Nutritional support, correction of dysphagia, gradual transition to independent nutrition
d330 Speech	During the 14 days course the patient will be able to make sounds, express basic emotional reactions and use alternative means of communication (gestures, facial expressions, communication cards) for conveying basic needs without using spoken language	33	23	Speech therapist	Enable voice and speech reactions in everyday situations
d240 Handling stress and other psychological demands	During the14 days course patient will reduce manifestations of anxiety and emotional instability, learn and apply minimum 2 self-regulation techniques with psychological support for reducing stress	22	11	Psychologist	Support, search and activation of resources, removal of tension

Table 3. Adaptation of environmental factors and examination of brain structures in the study cohort.

ICF codes and categories Domain «environmental factors» and «body structures»	Rehabilitation goals and objectives	Assessment		MDRT participant	IMRP
		O1 upon admission	O2 upon discharge		
e310 Immediate family	Within 14 days, ensure that the family is actively involved in the patient's care and rehabilitation, and enhance the knowledge and skills of family members in caring for tracheostomies and gastrostomies	+2	+3	PRM, psychologist, nurse	Informing, training in stoma care, and motivated counseling
e110 Products or substances for personal consumption	Ensure adequate and balanced nutritional and medication support	+2	+3	PRM doctor, consultants	Selection and correction of enteral formulas and medication therapy
s110 Structure of brain		3	3	PRM doctor, oncologist	Confirmation of remission/stable disease

most challenging cases, the following goals were achieved:

1) regained skills to climb independently and safely (under supervision) 5 flights of stairs; 2) ability to walk independently for least 100 meters with minimal fatigue; 3) ability to express basic emotional reactions using alternative means of communication (gestures, facial expressions, communication cards); 4) ability to convey simple needs without using voice speech; 5) ability to use voice and speech in everyday situations; 6) ability to eat independently. In all 9 cases, there was a reduction in anxiety and emotional instability.

There were no clinically significant complications in patients with tracheostomy and gastrostomy tubes during rehabilitation, thereby confirming the effectiveness of a multidisciplinary approach to medical rehabilitation based on high standards of patient care.

The systematization of rehabilitation goals according to the ICF main components clearly demonstrates a real multidisciplinary approach to management of stoma patients after neurosurgery at the stage of recovery from a state of minimal consciousness. Disrupted vital functions, such as breathing, swallowing, and speech control, are most clinically consequential, which is particularly noticeable in the domains of bodily functions and their corresponding domains of activity and participation.

Discussion

The rehabilitation of children after surgery for central nervous system tumors is associated with a number of challenges related to both, the underlying disease and the consequences of surgical and oncological treatment. Most demanding among all complications were cases of minimally conscious states associated with tracheostomy and/or gastrostomy. In this study, the proportion of such patients was only 2.9% confirming the exceptionality of such conditions. However, management of such patients requires significant resources and mandatory interdisciplinary coordination.

These data is consistent with the results of other studies, reporting the need for tracheostomy and gastrostomy in an average of 2–5% of children undergoing neuro-oncological treatment, especially when brain stem structures or cranial nerves are involved [7].

The results obtained in this study suggest that the organization of rehabilitation care for patients with CNS tumors does not differ in structure from that for surgical and palliative patients. However, there was a high demand for field-specific professionals, including an intensive care physician, ENT doctor, gastroenterologist, dietitian, and speech therapist, due to the complex nature of the disorders and the need for challenging management. The participation of an intensive care physician ensures adequate medical care for children in a state of minimal consciousness, taking into account the risk of possible life-threatening complications.

The need for regular sanitation and frequent (up to 2–3 times a week) replacement of tracheostomy tubes was due to potential proliferation of granulation tissue with the risk of obstruction and infectious complications, which is also mentioned in publications on the care of children in intensive care units [8]. In combination with swallowing disorders and feeding via a gastrostomy tube, this category of patients requires continuous monitoring of vital functions and a high level of medical competence on the part of the staff.

Recent studies emphasize the importance of objective monitoring of functional status, including autonomic nervous system activity, as one of the criteria for evaluating the effectiveness of therapy and rehabilitation in patients with severe brain damage [9]. This approach allows tracking the dynamics of vegetative regulation, which is especially important in patients with limited consciousness and lack of active feedback.

In addition to medical aspects, the emotional state of parents has a significant impact on the success of the rehabilitation process. Most family members of patients reported anxiety related to the duration of illness, the impairment of basic vital functions in the child, and uncertainty of the prognosis. Similar concerns are described in other studies, emphasizing the need for systematic psychological support for parents of children with severe functional impairments [10, 12]. The study conducted in the United States also reported on parents' concern about the fact that children with tracheostomies suffer not only from physical discomfort, but also from social isolation and inability to participate in normal school and play life [13].

In this regard, a clinical psychologist was involved in the rehabilitation activities to help parents regain emotional balance and build up relevant competence.

Conclusion

Uncommonness of the combination of CNS tumors with minimally conscious state in pediatric patients necessitates development of specialized multidisciplinary organizational and methodological solutions that take into account the phased tasks of cognitive function recovery to provide medical rehabilitation for this category of patients. The results obtained show that even in cases of severe functional impairments it is possible to achieve stabilization of the condition, partial recovery of lost functions, and improvement in the quality of life of both the child and the parents.

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Acute Methadone and Cocaine Poisoning Complicated by Cardiac Arrest: Case Report

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Summary

Objective: to evaluate the effects of correcting metabolic disorders in the post-resuscitation period in a clinical case report.

Materials and methods. Management and dynamic monitoring of acute poisoning in a 29-year-old patient after concomitant use of methadone and cocaine complicated by cardiac arrest and prehospital biochemical and acid-base balance alterations.

Results. The bradypneic and comatose patient developing out-of-hospital cardiac arrest (OHCA) was hospitalized after effective resuscitation by emergency team. Comprehensive lab examination revealed the presence of methadone and cocaine, decompensated metabolic lactic acidosis and hyperkalemia. Patient's condition improved after intense correction of metabolic alterations with sodium hydrocarbonate, a glucose-insulin mixture, and a pharmaceutical containing inosine + nicotinamide + riboflavin + succinate. A positive trend including recovered consciousness, switch from ventilator support to spontaneous breathing, and stable hemodynamics was documented after 3 days of treatment. However, emerging complications such as hospital-acquired pneumonia and acute kidney injury had to be managed. The patient improved significantly by the 17th day of treatment, and was discharged on day 21.

Conclusion. Intensive care to promptly address decompensated metabolic lactic acidosis (sodium hydrocarbonate, a multi-component drug containing inosine + nicotinamide + riboflavin + succinate) and hyperkalemia (glucose-insulin solution), reduced the severity of metabolic alterations after cardiac arrest due to acute methadone and cocaine poisoning, favoring patient's outcome.

Keywords: acute poisoning; methadone; cocaine; cardiac arrest; sodium hydrocarbonate, succinate; Cytoflavin

Conflict of interest. The authors declare no conflict of interest. NTFF POLYSAN LLC did not initiate the study and had no influence on the study design, analysis of the obtained data, interpretation of the results and writing the manuscript.

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Introduction

Currently, cardiac arrest assignable to various causes remains an urgent problem in resuscitation due to high rate of disability and fatal outcomes [1, 2]. According to published data, the survival rate in out-of-hospital cardiac arrest (OHCA) does not exceed 7–10% [3, 4]. Acute poisoning with the opioid drug methadone is the most common cause of death among all poisoning — associated OHCA cases. According to the Center for acute poisoning at the I. I. Dzhanelidze Research Institute of Emergency Medicine (St. Petersburg), in 2022, acute

methadone poisoning accounted for 38.5% of all poisoning-related deaths, in 2023, it was 42.4%, and in 2024 — already for 40%. In addition to the specific mechanisms of toxicant damage, emerging severe metabolic disorders significantly exacerbate the clinical course of poisoning [5], eventually resulting in fatal outcomes of chemical injury [6]. Polydrug poisoning following simultaneous use of narcotic substances with conflicting mechanisms of action, for example, with depressing and stimulating effects (pendulums) should be mentioned as very dangerous and unfavorable in terms of outcomes. According to our data, recently there has been an increase in acute poisoning with mixtures of methadone and other psychostimulant narcotic substances, however, in available literature we failed to find info on specific clinical features of such poisonings.

Methadone (6-dimethylamino-4,4-diphenyl-3-heptanone) is a synthetic substitute for opioid alkaloids (a synthetic opioid) [6]. Cocaine belongs to the group of psychoactive and sympathomimetic stimulants of the central nervous system [7, 8].

Presented clinical case could be of interest for specific intensive care management of acute severe poisoning by a mixture of narcotic substances with conflicting mechanisms of action (depressant toxicant methadone and stimulant toxicant cocaine), complicated by the development of OHCA.

Timely initiated extended cardiopulmonary resuscitation in the ambulance and continued in the hospital included chest compressions, mechanical ventilation, intravenous administration of epinephrine, infusion therapy, and syndrome-specific therapy. Measures were also taken to reduce mixed decompensated metabolic acidosis, hyperkalemia, and provide pharmacological neuroprotection using a multicomponent pharmaceutical containing inosine + nicotinamide + riboflavin + succinate (Cytoflavin, POLISAN LLC).

Objective: to evaluate the effects of correcting metabolic disorders in the post-resuscitation period in a clinical case report.

Results

Patient K., 29 years old, was admitted to reanimatology and intensive care department No. 11 (toxicology) (ICU No. 11) of the I. I. Dzhanelidze Research Institute of Emergency Medicine on February 14, 2023, at 02:48, in a critical condition.

Medical history: according to the data provided by the ambulance doctor, the patient was found at home with impaired (very close to coma) consciousness, severe bilateral non-reactive miosis and severe respiratory depression with 2–3 breaths per minute and a $SPO2_2$ level of 20% at the time of initial medical examination. Based on these findings the emergency medical team doctor diagnosed «opioid syndrome» (as depressed consciousness, bradypnea, and miosis are specific characteristics of acute opioid drug poisoning). The presence of an insulin syringe near the patient and a post-injection mark on the left upper limb were additional confirmation of the diagnosis of acute opioid poisoning. Antidote naloxone was not administered by the ambulance team.

Five minutes after tracheal intubation and initiation of mechanical ventilation, the patient experienced a cardiac arrest, and the ECG showed asystole. The ambulance team performed extended cardiopulmonary resuscitation (CPR), including chest compressions, continuous mandatory ventilation (CMV mode; FiO_2 — 100%, PEEP — 5 cm of water), administration of epinephrine 1 mg bolus each 3 minutes No. 3 and 12 mg dexamethasone, infusion of glucose-insulin mixture and 10 ml of multicomponent drug containing inosine + nicotinamide + riboflavin + succinic acid. The sinus rhythm was restored after 10 minutes of resuscitation, and dopamine was administered at a rate of 10 μg/kg/min.

An extremely severe condition on admission to ICU No. 11 resulted from severe toxic-hypoxic encephalopathy, clinically manifested by deep atonic coma (3 scores on the Glasgow Coma Scale (GCS), and acute respiratory failure (ARF) necessitating mechanical ventilation, with acute circulatory failure, which was treated with dopamine at a dose of 10 μg/kg/min. Inotropic and chronotropic effects of dopamine were acting on cardiotoxic effects of methadone, manifested as bradycardia and hypotension. Chemical and toxicological examination using gas chromatography-mass spectrometry revealed methadone and cocaine in the patient's urine.

Laboratory tests showed significant increase in the white blood cell (WBC) count (23.94×10^9/l, reference range $4.0–9.0\times10^9$/l). Biochemistry panel revealed an increase in creatine kinase to 1794 units/L (reference 7.0–190.0 units/L) and creatinine to 275 μmol/L (reference 60.0–120.0 μmol/L), indicating systemic rhabdomyolysis and acute kidney injury. Upon admission, serum concentration of myoglobin was 84 μg/L (reference 23.0–72.0 μg/L). Analysis of blood plasma electrolytes revealed severe hyperkalemia, reaching up to 6.14 mmol/L (reference 3.5–5.0 mmol/L). The arterial blood gas (ABG) measurements showed altered acid-base balance (ABB) due to hypoxemia and decompensated metabolic lactate acidosis: pH — 6.98; pCO_2 — 37.6 mmHg, pO_2 — 69 mmHg (with a fraction of inhaled oxygen (FiO_2) 80%), SO_2 — 86.1%, HCO_3 — 8.6 mmol/l, BE (base excess) — –23 mmol/l, arterial blood lactate — 19.8 mmol/l (reference 0.6–1.4 mmol/l). A low value of the Horowitz index — 86.3 mmHg confirmed impaired

transpulmonary oxygen transport and inadequate gas exchange function.

Chest X-ray showed enhanced pulmonary pattern on admission.

The patient's diagnosis on admission was as follows: «Severe acute poisoning with a mixture of narcotic substances (methadone, cocaine). Toxic-hypoxic encephalopathy. Coma stage 3. Complications: Acute respiratory failure. Acute circulatory failure. Cardiac arrest on February 14, 2023. Systemic rhabdomyolysis. Acute kidney injury».

In the hospital, the patient was managed on mechanical ventilation in backup modes under continuous ABG and ABB monitoring and dopamine support at a dose of 10 μg/kg/min. Decompensated metabolic acidosis was corrected by administering 4% sodium bicarbonate. The amount of required bicarbonate was calculated using the Mellengaard–Astrup equation [9]. According to calculation, the amount of required hydrocarbonate was 552 mmol, and 2000 ml/day of glucose-insulin mixture (12 IU of insulin added to each 500 ml of 10% glucose) was infused to correct hyperkalemia. A multi-component pharmaceutical Citoflavin (inosine, nicotinamide, riboflavin, and succinic acid) at a dose of 10 ml per each 500 ml was added to the glucose-insulin solution to provide neurometabolic support and decrease the lactate level. The therapeutic regimen also included proton pump inhibitors (omeprazole lyophilisate at a dose of 40 mg dissolved in 100 ml of 5% glucose solution, administered intravenously over a period of 30 minutes once daily), thiamine 50 mg administered intramuscularly once daily, and continuous intravenous infusion of heparin Na at a rate of 10 IU/kg/h during patient's management in the ICU. To interrupt enterohepatic circulation of methadone, the patient was subjected to gastric lavage and cleansing enema, administered enterosorption and laxatives (Duphalac 50.0 ml).

In view of deterioration caused by progression of circulatory failure 3 hours after admission, the patient's dose of dopamine was increased to 15 micrograms/kg/min. However patient's toxic-hypoxic encephalopathy and impaired consciousness persisted, assessed as grade 3 coma (GCS 3). Continuous infusion therapy improved baseline metabolic alterations, allowing de-escalation of the dopamine support dose to 7 μg/kg/min after 4 hours, and to 5 μg/kg/min after 12 hours of infusion.

Stabilization of systemic hemodynamic parameters was achieved 24 hours after the initiation of treatment, and dopamine support was discontinued. Toxic-hypoxic encephalopathy has also diminished allowing patient's consciousness to improve to the level of stupor (GCS 10 scores). A simultaneous decrease in WBC count to 11×10^9/l was also documented. Positive shifts in ABG and ABB were also evident, based on lab tests data, including: pH = 7.341, pCO_2 = 60.2 mmHg, pO_2 = 110.9 mmHg, SO_2 = 97.9%, HCO_3^- = 31.8 mmol/L, BE = 4.7 mmol/L, with a fraction of inhaled oxygen (FiO_2) of 40%, and a decrease of potassium to 3.7 mmol/L. There was also a decrease of arterial blood lactate to 3.2 mmol/L and an improvement in pulmonary gas exchange function (an increase in the Horowitz index to 275 mmHg). However, biochemistry panel indicated to deterioration of kidney function, i.e. progression of impaired nitrogen excretion, resulting in increases of creatinine to 337 mmol/l (reference 60.0–120.0 mmol/l), and urea — to 20.3 mmol/l (reference 0–8.3 mmol/l); worsening of cytolysis syndrome evidenced by aspartate aminotransferase increase to 6844.5 u/l (reference is up to 31 u/l) and systemic rhabdomyolysis (an increase of creatine kinase to 10545 u/l (reference is 7.0–190.0 u/l) and myoglobin to 7856 mcg/l).

A relative decrease in daily urine output to 1000 ml/day was documented along with above-mentioned lab findings compared to daily infused volume of 3200 ml, which was associated with the progression of acute kidney injury due to escalating systemic rhabdomyolysis. To remove circulating myoglobin, a hemodialysis was administered using membranes with a high cutoff point and the following parameters: perfusion rate of 300 ml/min, ultrafiltration rate of 1.0 L/h, dialysate flow of 500 ml/min, sodium conductivity of 140 mmol/l, perfusion time of 240 min, 72 L of blood were processed, 4.0 L of fluid were removed, and the fluid deficit was 3.0 L. Lab test performed 6 hours after hemodialysis showed decrease of creatine kinase to 3348 U/L and of myoglobin — to 3153 μg/L. On day 6 of intensive care including 3 hemodialysis sessions the concentrations of creatine kinase decreased to 263 U/L and of myoglobin — to 81 μg/L. Symptoms of encephalopathy resolved by the third day, the patient recovered spontaneous breathing and was extubated. However, new complications emerged on the same day, including in-hospital ventilator-associated pneumonia requiring administration of antibacterial therapy, and deterioration of acute kidney injury, necessitating five sessions of hemodialysis, after which renal function recovered on the 17th day, as evidenced by a decrease of creatinine to 194 μmol/L and urea to 8.9 mmol/L.

The patient was discharged from the hospital on the 21st day in a satisfactory condition.

Therefore, effective resuscitation measures provided by the emergency medical team during prehospital care alongside with effective correction of metabolic disorders (decompensated metabolic lactic acidosis, hyperkalemia, and hyperlactatemia), and the use of hemodialysis in the ICU setting resulted in favorable outcome for the patient experiencing acute poisoning with a mixture of narcotic

substances (methadone, cocaine), complicated by cardiac arrest.

Discussion

The available published data on acute poisoning with a mixture of narcotic substances containing opioids and cocaine only allows to conclude that such a combination is extremely unfavorable, and clinical course of concomitant poisoning is understudied [10–12]. Therefore, this clinical case is interesting in terms of pathogenesis of both — the critical state, including acute respiratory failure, cardiac arrest and coma due to acute methadone and cocaine poisoning, and the recovery from it owing to adequate intensive care in the ICU setting.

One of major causes of cardiac arrest was unfavorable combination of narcotic substances with conflicting mechanisms of action: the methadone depressant effect on the respiratory center and cardiovascular system leading to bradypnea and bradycardia, and the stimulating effect of cocaine, which provokes tachycardia, arterial hypertension, and an increase in myocardial oxygen demand. In our opinion, this pathophysiological «conflict» was one of the leading causes of OHCA.

Systemic rhabdomyolysis due to severe hypoxia caused by methadone-induced respiratory depression, which further led to severe mixed respiratory and metabolic acidosis could also contribute to circulatory failure [13], enhanced by psychostimulant effect of cocaine, which increased tissue oxygen demand due to the direct cytotoxic effect of cocaine on skeletal muscles [14].

Moreover, massive damage to muscular tissue was another factor that exacerbated decompensated metabolic acidosis and hyperkalemia, which were also key factors leading to cardiac arrest. After successful resuscitation outside of a medical facility, the patient developed acute kidney injury, which had both a pre-renal component (due to circulatory arrest), and a renal component caused by myoglobinuria due to systemic rhabdomyolysis [13, 14]. This determined the need for renal replacement therapy as an integral part of the patient's management leading to recovery.

It should be noted that the medical interventions not only saved the patient's life but also minimized the consequences of the circulatory arrest. Firstly, the timely and effective resuscitation measures outside the hospital laid the foundation for further successful treatment. Secondly, rapid correction of metabolic disorders such as decompensated metabolic acidosis (using sodium bicarbonate), hyperkalemia (using a glucose-insulin mixture), and lactatemia (using a multi-component drug: inosine + nicotinamide + riboflavin + succinic acid).

It should be especially noted that the routine use of sodium hydrocarbonate is not recommended currently, according to guidance of the European Resuscitation Council from 2021 [15] and the American Heart Association from 2021 [16]. However, the exceptions to recommended algorithm in notes to item 25 in «Cardiac Arrest» (Adult Patients) [17] clinical guidelines include cardiac arrest in acute opioid drug poisoning, so the use of sodium hydrocarbonate was one of the basic components of treatment. In our opinion, the use of a multicomponent drug (inosine + nicotinamide + riboflavin + succinic acid) in the acute stage significantly reduced the energy deficiency of brain cells, which had a positive effect on brain functions recovery in the post-resuscitation period [18, 19].

Conclusion

This clinical case demonstrated the effectiveness of a comprehensive approach to the intensive care of a patient with combined acute poisoning by drugs with conflicting mechanism of action (methadone, cocaine), complicated by cardiac arrest.

The main factors contributing to a favorable outcome were timely and high-quality advanced resuscitation measures implemented at the prehospital stage and in the ICU setting; correction of life-threatening metabolic disorders (lactic acidosis, hyperkalemia); active detoxification; and pathogenetic therapy aimed at correcting energy metabolism.

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A Confidential Questionnaire on Perioperative Critical Incidents as a Tool for Evaluating Anesthesia Safety Problems: a Pilot Study

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Summary

There is a general agreement on the need to analyze critical incidents in the operating room (OR) in order to develop effective ways to predict associated fatal consequences, as well as to develop decision support cognitive tools that can be used in both routine and crisis situations.

Research objective. Developing and testing of a confidential questionnaire on perioperative critical incidents by anaesthesiologists and other intensivists, assessment of the initial results of the tool application including its possible relevance to further implementation in the Russian Federation health care

Materials and methods. The survey was conducted by anonymous questioning among doctors of large hospitals in St. Petersburg from 01.06.2024 to 31.12.2024 using an on-line form.

Results. Of the 32 questionnaires received , 27 (84.4%) were completed and contained complete information, which was systematized and analyzed. Respondents reported most often on critical incidents in male patients (15 patients, 55.6%), and in the age group of 45–59 years (8 cases, 29.6%). Most of the incidents occurred during minimally invasive video assisted surgery (18 cases, 66.7%). In 14 cases (51.8%), critical incidents were related to human error, and in 9 (33.3%) cases, they were related to unexpected complications in the perioperative period. Most of the respondents (20 out of 27, 74.1%) noted that the incidents could have been prevented with more accurate anesthesia monitoring and relying on more sophisticated and performant anesthetic equipment in the Intensive Care Unit. Doctors positively evaluated the trial version of the anonymous questionnaire (22 out of 27, 81.5%), noting its simplicity and usefulness in identifying problems. Several respondents suggested adding open-ended questions to provide a more detailed description of the incidents.

Conclusion. The study confirmed the usefulness of designing and implementing a national questionnaire on perioperative critical incidents in Russia. The data obtained demonstrate that such a tool, combined with improving the staff training, equipment upgrade, and standardization of protocols, can significantly contribute to enhancing patient safety in anesthesia. Ensuring the confidentiality of the provided data is crucial for obtaining statistically significant information. The development, implementation, and expansion of the proposed tool's database under the auspices of the Federation of Anesthesiologists and Resuscitation Specialists on a regular basis will undoubtedly contribute to improving the quality and safety of anesthesiology and resuscitation services in Russia.

Highlights

— 76.5% of respondents believe that incidents can be prevented by improving anesthesia control and OR anesthetic equipment.

— 81.5% of the surveyed specialists positively assessed the need for implementing the questionnaire, as well as its simplicity and anonymity.

Keywords: confidential questionnaire; perioperative critical incidents; anesthesia safety; anesthesia; perioperative period

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Introduction

Modern anesthesiology has made significant progress in ensuring patient safety, but perioperative critical incidents related to anesthesia continue to be a significant concern [1]. These incidents require ongoing analysis and development of new risk management strategies [2, 3]. One key tool in this regard is a national confidential questionnaire on perioperative critical incidents, which can be used to systematically collect and analyze data provided without regard to the «esprit» of particular healthcare professionals and medical organizations, in order to create effective strategies for preventing adverse events [4].

The relevance of introducing such a questionnaire is due to several factors. Firstly, perioperative critical incidents, such as respiratory and hemodynamic disorders, remain the leading causes of anesthesia-related deaths [5]. Secondly, such common risk factors, as surgery in non-working hours and high anesthetic risk according to ASA (American Society of Anesthesiologists) classification, continue to have a significant impact on safety of anesthesia [6–9]. Thirdly, concerns about the institution's reputation and one's own credibility often prevent colleagues from reporting objective information without anonymizing it [6, 10, 11].

It is important to note that despite reduction of anesthesia-related mortality, the main causes of anesthetic incidents remain unchanged [7–9, 12, 13]. This highlights the need for continuous monitoring and analysis of the weaknesses in established protocols, methods, and working practices. A national questionnaire can be an important tool in this process, providing a standardized approach to collecting data on critical incidents.

Regular use of the national questionnaire grants the important advantage of systematic collection of data, which allows for an objective estimate of the rate and nature of perioperative incidents. Further analysis of risk factors helps to identify the main causes of complications and develop measures to prevent them. By using the processed data, it is possible to create effective risk management tools, as well as develop and implement practical protocols and recommendations.

No doubt, the development and implementation of such a questionnaire require coordination at levels of the professional community of anesthesiologists, medical educational institutions, and large and small hospitals.

The aims of the study included developing and testing of a confidential questionnaire on perioperative critical incidents by anesthesiologists and other intensivists, assessment of the initial results of its application, including the relevance of such tool implementation in the Russian Federation health care.

Research objectives:

1. To ensure the confidentiality of data from both the respondents and the patients, in order to obtain the most reliable and objective data, which would allow anesthesiologists and intensivists to share their experiences without any concerns.

2. Based on the respondents' comments, develop a structured questionnaire that will cover all key aspects of the perioperative period, including patient preparation, choice of anesthesia type and method, anesthesia induction, intraoperative manipulations, and monitoring results, as well as the early postoperative period (up to 24 hours).

3. Analyze all data on the frequency and nature of perioperative critical incidents to identify the main contributing causes and factors.

4. Assess this tool in terms of feasibility and necessity for its further implementation in the Russian Federation.

5. Identify perspectives and obstacles for the widespread implementation of the questionnaire at the national level.

Materials and Methods

The survey by anonymous questionnaire was conducted in multidisciplinary hospitals in St. Petersburg from June to December 2024 among anesthesiologists and intensivists to comprehensively assess perioperative critical incidents.

Data collection was implemented through the online platform Google forms, which ensured the standardization of the process, the respondents' privacy and the convenience of filling out the questionnaire. Special emphasis was given to ethical aspects, all answers were completely anonymized, personal data of participants and patients were not collected and stored.

A structured questionnaire developed based on a literature review [14–19] and the experience of foreign research groups [4,20] was used as the main data collection tool. The questionnaire consisted of 4 sections covering all key aspects of the perioperative period:

1. Patient preparation: questions regarding preoperative examination, anesthesia risk assessment, and patient preparation for surgery.

2. Choosing anesthesia method: questions about the anesthesia techniques used and their effectiveness.

3. Monitoring during surgery: questions concerning methods of patient's vital functions monitoring and identifying critical incidents.

4. Recovery from anesthesia: questions concerning patient's recovery process and potential complications.

Each section contained clearly formulated questions that allowed for the collection of the most comprehensive information while maintaining the brevity of the questionnaire. Types of questions:

80% — closed-ended (multiple choice, Likert scales);

Fig. 1. QR code for accessing an anonymous questionnaire in GOOGLE Forms.

20% — open (for incident details).

To view the full version of the questionnaire, please use the QR code in Fig. 1.

IBM SPSS Statistics software version 29.0 (IBM Corp., USA) was used for statistical data processing. Categorical variables were described by absolute and relative frequencies (*N*, %). Continuous variables were checked for normality of distribution using the Shapiro–Wilk test. Data with a normal distribution were presented as the mean and standard deviation ($M \pm SD$), and non-normal distribution data were presented as the median and interquartile range [*Me (Q1; Q3)*]. Fisher's exact test was used to compare categorical variables due to the small expected number of observations in the table cells. Statistical significance of the differences was determined at a $P < 0.05$.

A comprehensive statistical analysis was used to process the data, including assessment of the occurrence rate of various types of incidents, identification of significant correlations between different factors and incidents, and a comparative analysis with international indicators of anesthesia safety (comparing the data obtained with the results of international studies on trends in anesthesia-related mortality).

Table 1. Demographic and clinical characteristics of patients experiencing critical incidents, *N* = 27.

Parameters	Occurrence rate of critical incidents, *N* (%)
Age, years	
Young,18–44	7 (25.9)
Average, 45–59	8 (29.6)
Elderly, 60–74	8 (29.6)
Senile, 75 and more	4 (14.8)
Gender	
Males	15 (55.6)
Females	12 (44.4)
Minimally invasive video-assisted procedures*	18 (66.7)
Assessment on the ASA scale **, classes	
I	5 (18.5)
II	13 (48.1)
III	7 (25.9)
IV	2 (7.4)

Note. * — the highest number of incidents was recorded during minimally invasive video-assisted procedures. ** — preoperative assessment of patient's physical status according to the ASA classification (American Society of Anesthesiologists).

Results

A total of 32 questionnaires were received. The following data necessary for classification of the incident were used as the inclusion criteria for the analysis: determining the nature of the incident, the period of its occurrence, and establishing the main cause. Questionnaires that lacked this essential information ($N = 5$) were excluded from the analysis. Therefore, the study included 27 questionnaires (84.4%) that contained complete data suitable for systematization.

The characteristics of patients who experienced critical incidents are presented in Table 1.

Common concomitant diseases are presented in Fig. 2.

Characteristics of the respondents. The majority of the respondents (24 specialists, 88.9%) had more than 3 years of work experience, which indicates the qualification and experience of the anesthesiologist.

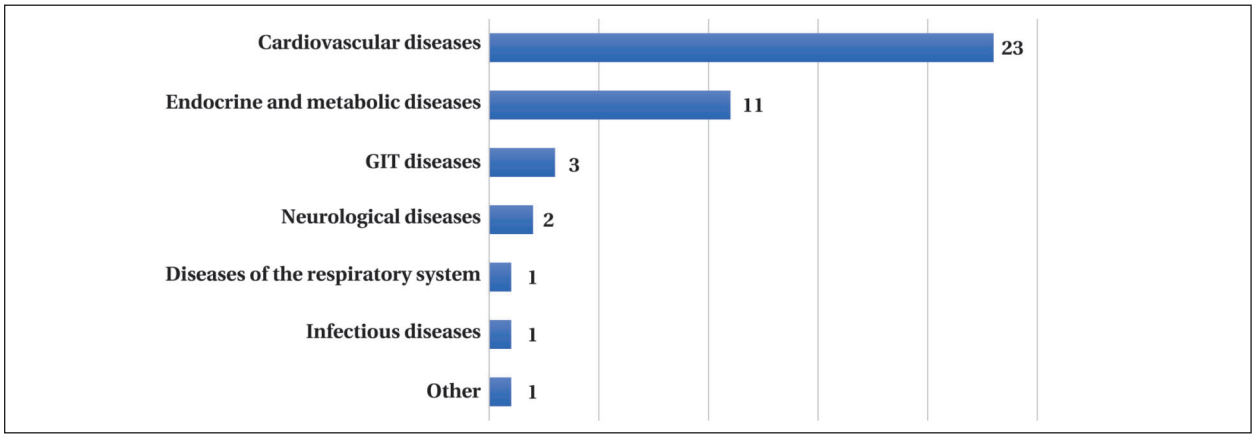

Fig. 2. Incidence of concomitant diseases in patients with critical incidents in the perioperative period.

Table 2. Perioperative critical incidents.

Incident Group	Specific events
Cardiac hemodynamic	Cardiac: cardiac arrhythmias, acute myocardial infarction (AMI), acute coronary syndrome (ACS)
Respiratory incidents	Bronchospasm, aspiration of gastric contents, difficult tracheal intubation, pneumothorax, hemo-pneumothorax
Non-cardiac hemodynamic	Hemodynamic: anaphylactic shock, hemorrhagic shock, massive blood loss
Neurological	High spinal block, transient ischemic attack, acute hemorrhagic cerebrovascular accident
Embolism and thromboembolic complications	Pulmonary thromboembolism (PTE), amniotic fluid embolism, and fat embolism
Technical and equipment specific	Gas line connection errors, respiratory circuit disconnection, and tracheal mucosal damage during tracheal intubation
Other	Malignant hyperthermia

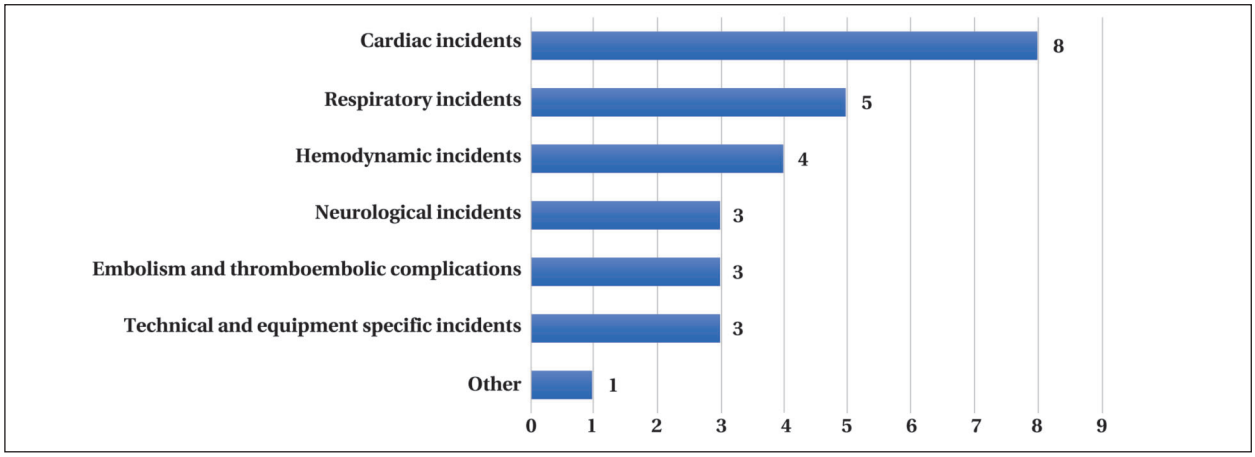

Fig. 3. Occurrence rate of critical incidents.

In 21 cases (77.8%), assistance from more experienced colleagues was available and provided.

The majority of respondents (20 people, 74.1%) worked in national-level hospitals, while the remaining 7 people (25.9%) worked in urban and regional medical facilities.

Occurrence rate of critical incidents. Data analysis showed that each of the respondents encountered with critical incidents in the perioperative period at least 2 times during their entire work experience. Fig. 3 shows the frequency of critical incidents in the analyzed data from the respondents.

Table 2 shows the distribution of events by category.

Meanwhile, 22 (81.5%) of the respondents noted that the incidents occurred during scheduled operations in the daytime (8:00–17:00), despite the fact that the patients and teams were prepared and all the risks seemed to have been taken into account.

Causes of incidents. According to the respondents, the main causes of critical incidents were:

1. Human error — 14 cases (51.8%), which includes: insufficient preoperative diagnostic elaboration and preparation, incorrect tactical decisions, errors in drug dosage calculation, incorrect interpretation of monitoring data, and medical malpractice during manipulations, including manipulations in an unequipped area.

2. Unanticipated challenges emerging during the procedure and not related to a medical error — 9 observations (33.3%), such as amniotic fluid embolism, thrombosis during joint replacement surgery, allergic reactions (to previously unestablished allergen), individual body characteristics and functional body reserves.

3. Equipment shortage — 2 cases (7.4% of cases), such as lack of modern diagnostic equipment, malfunction of anesthetic equipment (including a defibrillator), and disruption of the gas line connection.

4. Organizational issues — 2 observations (7.4% of cases), such as scheduling of overcrowded operating rooms and lack of time to adequately prepare for surgery, and the absence of a blood transfusion unit in the hospital.

At the same time, 20 (74.1%) respondents remarked that many incidents could have been avoided with more stringent monitoring throughout the conduct of anesthesia, sufficient anesthetist's experience, or the opportunity to rely on a more experienced colleague, more advanced facilities and equipment.

Evaluation of the questionnaire. The questionnaire was positively evaluated by 22 (81.5%) of

the respondents. They rated it as a high-quality tool for collecting data on critical incidents. They also noted its simplicity, anonymity, and its potential to identify key problems in the perioperative period. However, 8 (29.6%) of the doctors who completed the questionnaire indicated the need for further improvement of the questionnaire, particularly in terms of improving its intuitive clarity and precluding the possibility to dissimulate certain categories of questions when selecting a specific type of anesthesia.

The majority of the surveyed specialists (22 out of 27, 81.5%) positively assessed the need to implement the questionnaire, taking into account legal norms and law enforcement practices, on a regular basis in the Russian Federation, which would undoubtedly contribute to further improving the quality and safety of anesthesiological and intensive care through the analysis and publication of key factors leading to incidents, and systemic recommendations for their prevention.

Discussion

Systematic data collection on critical incidents using specialized questionnaires is an important tool for analyzing the causes of these incidents and developing preventive measures [12–14].

Analysis of the data revealed that critical incidents, including hypotension, hypovolemia, cardiac arrhythmias, circulatory arrest, hypoxia, and anaphylactic reactions, remain common, which is consistent with the results of international studies [9–17]. It is noteworthy that such incidents occur with equal frequency in patients of various age groups and are not always associated with severe concomitant pathologies, although the latter certainly worsen the prognosis.

Special attention should be paid to high frequency of critical incidents during video-assisted minimally invasive procedures (66.7%). This may be due to the specific conditions of such interventions, in particular, the need to reduce illumination in the operating room to improve visualization on the monitors. Such working conditions create additional difficulties, causing greater stress for the entire operating team, contribute to faster onset of fatigue and reduced concentration. Consequently, this increases the risk of various faults and flaws during both the surgery and the anesthesia, from technical equipment malfunctions to intraoperative complications.

According to the survey, other main causes of critical incidents include the human factor (51.8%) and unexpected perioperative events (33.3%). These results are consistent with findings from other studies, where the human factor and inadequate medical staff training are also leading causes of complications [1, 17–21]. Although organizational issues and inadequate equipment were mentioned less frequently, they still play a significant role, especially in resource-constrained settings [18].

The majority of respondents positively evaluated the proposed questionnaire, noting its advantages, such as ease of completion and guaranteed anonymity. However, the study participants emphasized the need for further improvement, particularly the inclusion of open-ended questions to provide a more detailed description of the incidents. This recommendation aligns with the findings of other studies aimed at enhancing data collection tools in healthcare [23]. Additionally, the incomplete submission of data in 15.6% of the questionnaires highlights the need for further refinement of the questionnaire structure and heightened motivation for respondents to provide more detailed information.

Refining the questionnaire based on the respondents' comments will make it more user-friendly and provide accurate and informative data about critical incidents, which will ultimately lead to the development of effective measures to improve the safety of anesthesia and surgical procedures in general.

When interpreting the results of this study, a number of limitations should be taken into account. The main limitation is the small sample size (N=27), which does not allow for a comprehensive multivariate statistical analysis to identify all significant relationships, and makes it impossible to statistically validate the questionnaire. The results are preliminary and descriptive in nature. Additionally, an important limitation is the lack of a formal assessment of the questionnaire's psychometric properties, such as reliability (internal consistency) and validity. This tool is designed to collect factual and categorical data on incidents and does not contain internal scales for which the Cronbach's alpha coefficient is typically calculated. The validation of such reporting tools focuses on content validity (the completeness of coverage of key aspects of the incident) and practical applicability, which was evaluated in this pilot study through feedback from respondents.

To successfully implement the questionnaire at the national level, it is necessary to undertake a series of actions. First of all, it is required to develop unified standards for data collection and analysis under the auspices of the Federation of anesthesiologists and intensivists. It is equally important to organize regular training for medical personnel aimed at minimizing errors related to the human factor [24]. Improving the basic infrastructure of medical facilities, especially in the context of providing emergency surgical care, also plays a significant role [24–26].

The use of anonymous questionnaires is an essential tool for ensuring security of healthcare

professionals when reporting incidents. In different legal systems, this methodology allows for the avoidance of criminal prosecution in countries where medical errors are criminalized, while also protecting against potential reputational risks and civil law consequences in countries with more liberal legislation. This approach creates the necessary conditions for open professional dialogue, facilitating the identification of systemic issues in healthcare without fear of legal consequences for participants [6, 10, 11, 21].

Sample expansion by including data from various regions of the Russian Federation looks like a promising area for further research. This will provide a representative picture and upgraded database for analyzing the accumulated information, along with identifying potential regional differences and developing preventive measures. This approach not only reduces the frequency of complications, but also promotes a safety culture that meets international WHO standards [25–27].

It is equally important to organize long-term monitoring of critical incidents using an improved questionnaire, as this approach allows for an objective assessment of the effectiveness of implemented preventive measures and tracking of changes over time. The systematic collection and comprehensive analysis of data on perioperative critical incidents contributes to improving patient safety and reducing the incidence of complications associated with anesthesia and surgical interventions.

Support for the development, testing, and validation of a national confidential questionnaire by the Russian Federation of anesthesiologists and intensivists could bring it closer to clinical practice.

Conclusion

The conducted study confirms the relevance of the developed questionnaire as a starting point for developing a promising tool for monitoring perioperative critical incidents. Despite the limited sample size ($N = 27$), the obtained data revealed important patterns.

It is crucial to identify the prevalence of preventable incidents related to human factors and technical aspects of medical care.

The willingness of the medical community to openly discuss critical situations is particularly valuable, as evidenced by the high percentage of respondents (22/27, 81.5%) who positively evaluated the anonymous survey format. To enhance the information content of the tool, it is advisable to include open-ended questions that allow for a more detailed analysis of the incident circumstances.

In this regard, despite the pilot nature of the study, the results obtained confirm the need to improve the questionnaire on a larger sample with multi-center participation of respondents.

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Providing Care for Developing Respiratory Failure During Mass Admission of Patients with COVID-19

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Summary

The COVID-19 pandemic has posed an unprecedented challenge to healthcare systems around the world. The mass influx of patients with severe hypoxemic respiratory failure, often progressing to acute respiratory distress syndrome (ARDS), has led to an acute shortage of beds in intensive care units (ICUs).

The aim of the study was to evaluate the effectiveness of an organizational model for the care of patients with severe COVID-19 pneumonia, including the creation of intensive observation wards (IOWs) for non-invasive respiratory support outside the ICU, and to develop a prognostic model for the risk of transferring patients to invasive mechanical ventilation (IMV) or non-invasive mechanical ventilation (NIMV).

Materials and methods. A retrospective observational study was conducted at the V. P. Demikhov city clinical hospital of the Voronovskoye Moscow clinical center for infectious diseases from January to December 2021. We analyzed data from 950 patients with confirmed COVID-19 and hypoxemic respiratory failure who started high-flow oxygen therapy (HFOT) in the IOW. The demographic structure, premorbid background, clinical and laboratory parameters, and respiratory and anti-inflammatory therapy regimens (glucocorticoids — GCS: dexamethasone or methylprednisolone, and/or monoclonal antibodies — MAbs) were studied. For 573 patients transferred from the IOW to the ICU, we assessed outcomes and risk factors for the need for NIMV/IMV using binary logistic regression.

Results. Of the 950 patients who started HFOT in the IOW, 573 (60.3%) were transferred to the ICU for escalation of respiratory support. The mortality rate in the ICU group was 25.7% (147 of 573 patients hospitalized in the ICU). When comparing GCS regimens with or without MAbs, the mortality rate in patients receiving methylprednisolone in any treatment regimen was lower than in patients receiving dexamethasone: 14.1% vs. 25.8% (with MAbs, $P < 0.001$) and 15.3% vs. 37.4% (GCS only, $P < 0.001$). According to the logistic regression model, predictors of increased risk for the need for NIMV/IMV were: older age (OR > 1.014 for each year [1.024; 1.058], presence of diabetes mellitus (OR > 1.530 [1.038; 2.2123]), and a higher NEWS score upon transfer to the ICU (OR > 1.342 for each score [1.153;1.562]). The use of methylprednisolone compared to dexamethasone was associated with a reduced risk of requiring NIMV/IMV (OR = 0.346 [0.238; 0.503]).

Conclusion. The organization of IOW for the implementation of HFOT according to a strict protocol made it possible to provide assistance to a large number of patients in conditions of ICU resource shortages. The use of methylprednisolone was associated with lower mortality in the ICU compared to dexamethasone. The developed prognostic model may be useful for stratifying the risks of escalating respiratory support methods and making timely decisions to transfer patients to non-invasive/invasive mechanical ventilation.

Keywords: acute respiratory failure; COVID-19; high-flow oxygen therapy; glucocorticoids; methylprednisolone; dexamethasone; prognostic model of risks of escalation of respiratory support; intensive care wards.

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

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Introduction

Humanity is constantly faced with epidemics and pandemics that claim thousands of lives. The effectiveness of medical and health care advances that made it possible to «tame» 19th century pathogens such as cholera led epidemiologists, medical historians, and other experts to believe that developed countries had managed to eradicate infectious diseases once and for all in the 20th and 21st centuries. As virologist Sir Frank Macfarlane (Mac) Burnet stated in 1951, «the eradication of infectious diseases has become a significant factor in the social structure». «To write about infectious diseases», he added in 1962, «is to stir up the long forgotten».

The COVID-19 pandemic caused by the SARS-CoV-2 virus has radically changed the landscape of modern medicine, posing unprecedented challenges for healthcare systems around the world [1, 2]. One of the most serious problems has been the massive influx of patients with severe lung damage, rapidly developing hypoxemic respiratory failure, and acute respiratory distress syndrome (ARDS) [3, 4].

Medical facilities around the world have faced enormous pressure on resources, especially in intensive care units (ICUs). A critical shortage of resuscitation beds, ventilators, and qualified personnel has necessitated an urgent reorganization of medical care and a search for new approaches to the management of patients with severe respiratory failure [5–7].

In response to these challenges, many hospitals adopted a strategy of creating intermediate levels of care — so-called intensive observation wards (IOWs) or respiratory support units — based in infectious disease or therapeutic departments. The main task of such units was to provide care to patients receiving ongoing pathogenetic therapy and standard low-flow oxygen therapy (LFOT) for respiratory disorders that do not meet the criteria for immediate transfer to mechanical ventilation but require escalation of respiratory support methods and closer monitoring of vital signs. The key method of respiratory support in IOW was high-flow nasal oxygen therapy (HFOT), which allows the delivery of heated and humidified oxygen-air flow at high speed and with a controlled oxygen fraction (FiO_2) [8, 9].

The effectiveness of this organizational model largely depended on a clear respiratory support protocol regulating both the indications for starting HFOT and the criteria for its effectiveness and the need for timely escalation of therapy — transferring the patient to the ICU for noninvasive mechanical ventilation (NIMV), invasive mechanical ventilation (IMV), or, in refractory cases, extracorporeal membrane oxygenation (ECMO) [10, 11].

Alongside respiratory support, the cornerstone of treatment for severe COVID-19 has been the suppression of the hyperinflammatory response (cytokine storm), which plays a key role in the development of ARDS and multiple organ failure. The main method of pathogenetic therapy was the administration of glucocorticoids (GCs), the effectiveness of which has been demonstrated in large international studies such as RECOVERY [12]. However, the optimal regimens, dosages, and choice of specific GCs drug (e. g., dexamethasone or methylprednisolone) remained a subject of debate and required study in real-world clinical practice [12, 13]. According to the temporary methodological recommendations and local protocols in force, various GCs regimens were used, including the use of dexamethasone or methylprednisolone [14–17]. In addition, interleukin inhibitors (monoclonal antibodies — MAbs) were used in some patients with signs of severe systemic inflammation [18–21].

In the context of mass admissions and limited ICU resources, assessing the effectiveness of the approaches used and early identification of patients at high risk of adverse outcomes or in need of escalated respiratory support became paramount. The development of prognostic models based on available clinical and laboratory data helps in risk stratification and optimization of patient routing [22–26].

The aim of the study is to evaluate the effectiveness of an organizational model for the care of patients with severe COVID-19 pneumonia, including the creation of (IOWs) for non-invasive respiratory support outside the ICU, and to develop a prognostic model for the risk of transferring patients to invasive mechanical ventilation (IMV) or non-invasive mechanical ventilation (NIMV).

Materials and Methods

A retrospective observational cohort study was conducted at the V. P. Demikhov city clinical hospital of the Voronovskoye Moscow clinical center for infectious diseases. The electronic medical records of patients admitted for treatment from January 1 to December 31, 2021, were analyzed.

Patients. During the specified period, 17,761 patients diagnosed with COVID-19 were hospitalized. 15,521 patients were sent to infectious disease wards, and 2,240 were sent directly to the intensive care unit (ICU), bypassing the emergency room. Of the total number of patients hospitalized in infectious disease wards, 3,053 (19.7%) were transferred to the ICU at various stages of treatment due to negative dynamics in their condition for various reasons.

Of the 15,521 patients, 950 (6.1%) were transferred to the IOWs of infectious disease departments for high-flow oxygen therapy (HFOT). As respiratory failure progressed, patients were transferred to the ICU for non-invasive mechanical ventilation (NIMV), invasive ventilation (IMV), or extracorporeal membrane oxygenation (ECMO).

The criteria for inclusion of patients in the study were: laboratory-confirmed diagnosis of

COVID-19; stay in the IOW/transfer to the ICU; age over 18 years; specific lung damage according to computed tomography (CT) data; presence of symptoms of hypoxemic respiratory failure requiring respiratory support with low-flow oxygen therapy (LFOT) or HFOT/NIMV.

Exclusion criteria: admission to the ICU bypassing the infectious diseases department; pregnancy of various stages; mental illnesses of various etiologies; transfer to the ICU for reasons not related to progressive lung tissue damage; acute surgical diseases.

The patient selection scheme for the study is presented in Fig. 1.

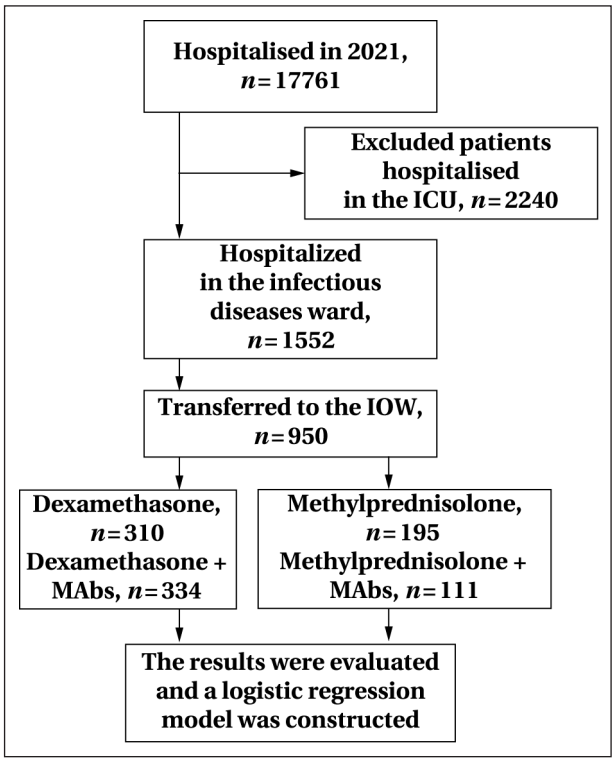

Fig. 1. Scheme of patient selection for analysis and construction of a logistic regression model.

Therapy. Patient examination, diagnosis of the underlying disease, its complications, concomitant diseases, and assessment of the severity of the patients' condition were carried out in accordance with the Temporary methodological recommendations (TMR) «Prevention, diagnosis, and treatment of the novel Coronavirus Infection (COVID-19)» versions 8 to 9, relevant at the time of the study.

Respiratory support was provided using a standard Bobrov system for oxygen therapy with a flow rate of 1–15 L/min (LFOT). High-flow oxygen therapy (HFOT) was used in patients with acute hypoxemic respiratory failure to deliver a humidified and warmed breathing mixture at a flow rate of 15–50 L/min and an oxygen fraction in the breathing mixture of 30 to 60% (AIRVO™ 2 Humidification System, New Zealand). Prone positioning (patient breathing in the prone position) was used for up to 16 hours per day as a second standard position. The third mandatory component was incentive spirometry using a Portex (Smiths Medical) Coach 2 exercise spirometer with a one-way valve and a volume of 4,000 ml, which was performed every 2 hours according to the standard method recommended by the manufacturer.

High-flow oxygen therapy was performed with the following parameters: oxygen flow in the air mixture 15–50 L/min, oxygen fraction in the respiratory mixture 40–60%. To assess the effectiveness of the therapy, the patient's clinical status (complaints, shortness of breath during exercise, respiratory rate) was monitored every 2 hours; SpO_2 while breathing atmospheric air — once a day; the goal of oxygen therapy was SpO_2 90–96%. The indication for transfer to the intensive care unit was the presence of one of the following criteria: SpO_2 during oxygen therapy < 92%, RR > 26 per minute, with maximum HFOT parameters of more than 50 L/min and FiO_2 > 60%, impaired consciousness (depression, agitation), hypotension (decrease in systolic blood pressure below 90 mm Hg).

Anti-inflammatory therapy was carried out with glucocorticoids alone or in combination with monoclonal antibodies. The first-line drugs for anti-inflammatory therapy in hospitalized patients with COVID-19 were glucocorticoids (GCs). The indication for the prescription of glucocorticoid hormones outside intensive care units was the development of signs of severe systemic inflammation and/or the progression of macrophage activation syndrome (increase in ferritin, serum C-reactive protein, development of dual/triple cell-line cytopenia). In such cases, one of the glucocorticoid drugs was used in the infectious diseases department:

— methylprednisolone (at a dose of 2 mg/kg/day intravenously, 1 mg/kg over an hour, then 1 mg/kg as a prolonged 24-hour infusion);

or

— dexamethasone (20 mg per day intravenously in two doses for at least 3 days, followed by a gradual dose reduction by 20–25% per dose every 1–2 days for 3–4 days, then by 50% every 1–2 days until complete withdrawal).

The indications for the use of monoclonal antibodies (tocilizumab, olucizumab) were: the presence of pathological changes in the lungs corresponding to CT 1–4 or moderate/severe pneumonia according to X-ray examination (infiltration-type diffuse opacities («white lung» symptom), involvement of ⩾50% of lung tissue) combined with two or more of the following signs:

— $SpO_2 \leqslant 93\%$, shortness of breath at rest;

— body temperature > 38°C for 5 days or recurrence of fever on the 5–10th day of illness after a «clear interval»;

— CRP ⩾ 9N or a 3-fold increase in CRP on the 8th-14th day of illness;

— white blood cell count < 3.0×10^9/L,

— absolute lymphocyte count < 1.0×10^9/L;

— blood ferritin ⩾ 250 ng/mL;

— IL-6 > 40 pg/mL.

Tocilizumab was administered at a dose of 4–8 mg/kg intravenously in combination with glucocorticoids (GCs).

If tocilizumab could not be used, an alternative regimen was considered that included the IL-6 receptor inhibitor levilimab 324 mg once (the contents of two pre-filled syringes of 162 mg/0.9 ml were diluted in 100 ml of 0.9% NaCl solution and administered intravenously by drip over 60 min). If the effect was insufficient, the administration was repeated after 12 hours [14, 20].

Statistical data processing was performed using the IBM SPSS Statistics v.27.0 software package. Quantitative data were presented as the arithmetic mean ± standard deviation (*M* ± *SD*) for normally distributed data, and as the median and interquartile range (*Me* [*Q1*; *Q3*]) for data with a distribution other than normal. Qualitative data were presented as absolute numbers and percentages (*n*, %). The normality of the distribution of the studied parameters was assessed using the Shapiro–Wilk test. Quantitative indicators between groups were compared using the Student's *t*-test or the Mann–Whitney *U* test, depending on the normality of the distribution of each parameter. Quantitative indicators were equalized using Pearson's χ^2 test or Fisher's exact test. Differences were considered statistically significant at $P < 0.05$. A two-tailed significance level was used.

The binary logistic regression method was used to determine the probability of NIMV/IMV use in patients and the prognosis of fatal outcome. This method calculates the probability of an event occurring depending on the values of independent variables.

Data from 950 patients were used to perform regression analysis.

The parameters taken as probable predictors of the use of NIMV or IMV were: gender (female — 0; male — 1), age (years), obesity (0 — no; 1 — yes), diabetes mellitus (0 — no; 1 — yes), concomitant cardiovascular diseases (0 — no; 1 — yes), NEWS scale score (scores), length of stay on HFOT (number of days), therapy (Dexamethasone — 0; Methylprednisolone — 1).

To construct the regression functions that make up the prognostic model, we used a multivariate analysis based on binary logistic regression with an adjusted OR estimate (taking into account the joint influence of predictors, corr. OR) and its 95% confidence interval. The predictors were introduced into the model stepwise backward (using Wald statistics), which made it possible to select informative predictors and exclude noisy ones.

To assess the quality of the logistic regression model, we used the Hosmer–Lemeshow test and calculated the percentage of total explained variance using the Nagelkerke method. Binary classification was evaluated based on ROC analysis.

The cutoff point for binarization was determined using the Judd criterion.

Descriptive statistics and hypothesis testing methods were used to analyze the data. Mortality was calculated as the percentage of deceased patients relative to the total number of patients in each group. The chi-square (χ^2) test was used to compare mortality between groups, which allowed to assess whether the observed difference in frequencies was statistically significant.

Results

Patient characteristics. In 2021, HFOT was initiated in 950 patients with COVID-19 pneumonia and hypoxemic respiratory failure in the IOW. The characteristics of this cohort are presented in Table 1.

Table 1. Characteristics of patients hospitalized in the IOW, *N* = 950.

Indicators	Indicator values
Gender	
Male	427 (44.9%)
Female	523 (55,1%)
Age, years	63.4 ± 10.8
Premorbid background	
Obesity	302 (31.8%)
Diabetes mellitus	237 (24.9%)
Cardiovascular diseases	731 (76.9%)
Duration of disease, days	6.02 ± 1.6
Degree of lung tissue damage according to chest computed tomography	
CT1 (less than 25%)	457 (48.1%)
CT 2 (25–50%)	393 (41.4%)
CT 3 (50–75%)	96 (10.1%)
CT 4 (> 75%)	4 (0.4%)

Patients mean age was 63.4 years, with a predominance of women (55.1%). The vast majority (76.9%) had concomitant cardiovascular diseases (CVD). Obesity and diabetes mellitus were found in 31.8% and 24.9% of patients, respectively. HFOT was intiated on average on the 6th day of illness. In most patients, the degree of lung damage on CT corresponded to CT 1–2 (89.5%) at baseline.

Transfer to the ICU and outcomes. Despite the therapy provided in the IOW, 573 patients (60.3% of those receiving HFOT in IOW) were transferred to the ICU for escalation of respiratory support using HFOT with a flow rate > 50 L/min, an inspiratory oxygen fraction > 60%, and NIMV/IMV. The characteristics of patients transferred to the ICU are presented in Table 2.

Among patients transferred to the ICU, women were older than men (68.1 vs. 62.2 years), and they were more likely to have obesity (42.1% vs. 30.9%) and CVD (86.8% vs. 81.4%).

Table 2. Characteristics of patients transferred from the IOW to the ICU .

Parameter	Men, $N=269$	Women, $N=304$	Total, $N=573$
Age, years ($M \pm SD$)	62,17 ± 10,94	68,1 ± 10,94	65,3 ± 11,3
Premorbid background			
Obesity, *n* (%)	83 (30,9)	128 (42,1)	211 (36,8)
DM, *n* (%)	76 (28,3)	100 (32,9)	176 (30,7)
CVD, *n* (%)	219 (81,4)	264 (86,8)	483 (84,3)
Duration of illness before transfer to the ICU, days ($M \pm SD$)	6,12 ± 1,76	5,97 ± 1,74	6,04 ± 1,75
Need for NIMV/IMV, *n* (%)	108 (40,1)	131 (43,1)	239(41,7)
(NIMV/IMV)	(37/71)	(43/88)	(80/159)
Mortality in the ICU, *n* (%)	64 (23,8)	83 (27,3)	147 (25,7)

Table 3. Mortality among ICU patients depending on the anti-inflammatory therapy regimen used.

Treatment regimens	Number of patients, *n* (%)		
	By treatment regimen	Transferred to ICU	With fatal outcome in the ICU
[1]Dexamethasone only	310	175 (56.5)	65 (37.1)
[2]Methylprednisolone only	195	118 (60.5)	18 (15.3)
$p_{1,2}$		0.368	< 0.001*
[3]Dexamethasone + MAb	334	209 (62.6)	54 (25.8)
[4]Methylprednisolone + MAb	111	71 (63.9)	10 (14.1)
$p_{3,4}$		0.793	< 0.001*

Note. MAb — monoclonal antibodies. *p* — level of statistical significance calculated using the χ^2 criterion. * — values of $P < 0.001$ indicate a statistically significant reduction in mortality with methylprednisolone therapy, both as monotherapy and in combination with MAbs, compared with similar use of dexamethasone.

Table 4. Prediction of the need for NIMV/IMV in the ICU based on the results of binary logistic regression ($n=573$).

Predictor	Coeff.	Standard error	Wald stat.	*p*	Exp (B) (corr. OS)	95% CI for OS, boundaries	
						lower	upper
Age	0.040	0.008	22.505	< 0.001	1.041	1.024	1.058
Gender	0.387	0.187	4.304	0.038	1.473	1.022	2.123
Diabetes mellitus	0.426	0.198	4.607	0.032	1.530	1.038	2.257
CVD	0.758	0.336	5.092	0.024	2.134	1.105	4.124
NEWS scale	0.294	0.077	14.422	< 0.001	1.342	1.153	1.562
GCs therapy	–1.061	0.191	31.006	< 0.001	0.346	0.238	0.503
HFOT	0.101	0.016	37.728	< 0.001	1.106	1.024	1.142
Constant	–6.770	0.762	78.927	< 0.001	0.001	—	—

The overall mortality rate in the ICU was 25.7%, with no statistically significant differences between men (23.8%) and women (27.3%).

Use of anti-inflammatory therapy and outcomes in the ICU. All patients in the ICU were prescribed GCs. In 444 (77.5% of those transferred to the ICU, or 46.7% of those receiving HFOT in IOW), MAbs were additionally used in treatment.

Rate of fatal outcomes, depending on the anti-inflammatory treatment regimen used: GCs monotherapy or in combination with MAbs is presented in Table 3.

It was found that when using methylprednisolone (both as monotherapy and in combination with MAbs), mortality among patients transferred to the ICU was statistically significantly lower than when using dexamethasone (15.3% vs. 37.1% for GCs monotherapy, $P=0.0001$; 14.1% vs. 25.8% for GCs+MAb combination, p=0.046). The addition of MAb to dexamethasone statistically significantly reduced mortality (37.1% to 25.8%, p=0.0117), while with methylprednisolone, the addition of MAb did not result in a statistically significant change in mortality (15.3% vs. 14.1%, p=0.823) in this cohort of ICU patients.

Predictive model of the need for NIMV/IMV. To identify factors associated with the need for NIMV or IMV in patients transferred to the ICU ($n=573$), a binary logistic regression model was constructed. After stepwise selection using backward elimination, the following predictors were included in the final model: age, presence of diabetes mellitus, NEWS score upon transfer to the ICU, and type of GC used (methylprednisolone vs. dexamethasone). The results are presented in Table 4.

The model showed good predictive power. The Hosmer–Lemeshow test was insignificant ($\chi^2 = 0.309$, df = 8, $P > 0.05$), indicating good model calibration and a fairly accurate description of the actual data. Nagelkerke's pseudo-R^2 was 0.255, meaning that the model explains 25.5% of the variance in the dependent variable. The area under the ROC curve (AUC) was 0.792 (95% CI: 0.758–0.827; $P < 0.001$), indicating high discriminatory power of the model (Fig. 2). The optimal cutoff point, determined by the Youden index, was 0.197, which corresponds to a sensitivity of 75.7% (95% CI: 68.8–81.7%) and a specificity of 70.7% (95% CI: 67.3%–73.9%) for predicting the need for NIMV/IMV.

The regression equation is as follows:

Z = 0.040 × Age + 0.387 × Gender + 0.426 × Diabetes mellitus + 0.758 × CVD + 0.294 × NEWS scale-1.061 × Therapy + 0.101 × HFOT – 6.770

According to the model, the predictors of an increased risk of requiring NIMV/IMV were: older age (OR > 1.014 for each year [1.024;1.058], gender, presence of diabetes mellitus (OR > 1.530 [1.038; 2.2123]), and a higher NEWS score upon transfer to the ICU (OR > 1.342 for each score [1.153; 1.562]). The use of methylprednisolone (compared to dexamethasone) was associated with a reduced risk of requiring NIMV/IMV (OR = 0.346 [0.238; 0.503]). Gender, obesity, CVD, and duration of ICU stay had a lower weight as independent predictors in the final model.

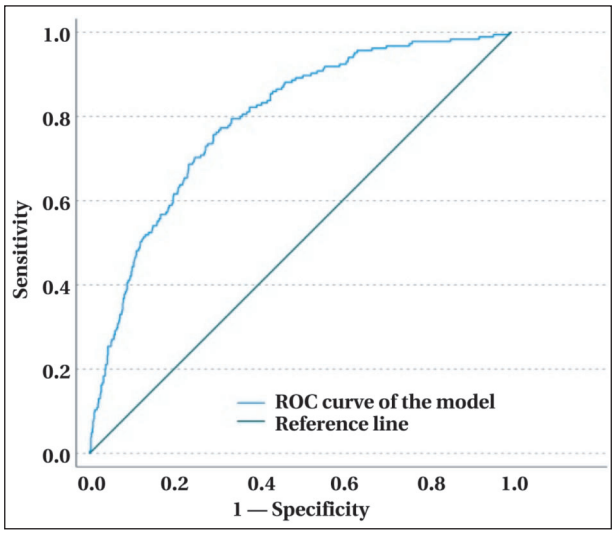

Fig. 2. ROC curve for the predictive model of the need for NIMV/IMV.

Discussion

We presented our experience in organizing medical care for patients with severe COVID-19 in the context of significant healthcare system overload in 2021. Faced with a shortage of intensive care beds, our hospital implemented a model of stepwise respiratory support, including the active use of HFOT in specially organized IOW outside the ICU.

The data obtained indicate that this strategy made it possible to provide care to a large number of patients (950 people started HFOT in the IOWs), but a significant proportion of them (60.3%) needed to be transferred to the ICU for escalation of respiratory therapy. The high frequency of transfers reflected the severity of COVID-19-associated respiratory failure during the pandemic waves studied and highlights the importance of clear criteria for timely escalation of treatment. However, it can be assumed that without the HFOT stage in the IOW, the burden on the ICU would have been even higher, and some of the patients successfully treated in the IOW (about 40%) could have occupied ICU beds.

The overall mortality rate among patients transferred to the ICU was 25.7%, which is comparable to data from other studies of that period for patients with severe COVID-19 requiring intensive care [15, 16]. Demographic characteristics and the presence of comorbidities (high incidence of CVD, diabetes mellitus, obesity) also correspond to global data on risk factors for severe COVID-19 [17].

One of the key aspects of our study was to evaluate the impact of different GCs regimens on outcomes in the ICU. The results indicate a statistically significant lower mortality rate in patients receiving methylprednisolone at a dose of 1 mg/kg/day (as a bolus and prolonged infusion) compared to patients receiving dexamethasone 20 mg/day. This difference persisted both with GCs monotherapy and in combination with MAbs. Moreover, the use of methylprednisolone was associated with a reduced risk of requiring NIMV/IMV according to logistic regression data. These results are consistent with data from several other studies suggesting potential advantages of methylprednisolone over dexamethasone in severe COVID-19 ARDS, possibly due to a more pronounced anti-inflammatory effect or better penetration into lung tissue [10, 11, 18]. However, it is important to note the retrospective nature of the analysis, which does not allow us to exclude the influence of unaccounted factors and bias in the choice of therapy. Nevertheless, the data obtained support the hypothesis of the potential advantages of the methylprednisolone regimen used and require further confirmation in prospective studies.

Interestingly, the addition of MAbs to GCs was associated with a reduction in mortality only in the dexamethasone group, but not in the methylprednisolone group. This may indicate that when using a more intensive GCs regimen (methylprednisolone 1 mg/kg/day), the additional effect of IL-6 inhibition may be less pronounced or may only be evident in a specific subgroup of patients. This observation also requires further study.

The developed prognostic model for the need for NIMV/IMV in the ICU demonstrated good predictive ability (AUC > 0.792). The identified predictors — age, presence of diabetes mellitus, and severity of condition on the NEWS scale at transfer — are known risk factors for unfavorable COVID-19 outcomes [13, 17]. The inclusion of GCs entity (methylprednisolone as a protective factor) in the model further emphasizes the potential impact of the choice of anti- inflammatory therapy on the course of the disease. This model can be used in clinical practice for the early identification of patients at high risk of requiring invasive respiratory support, allowing for the timely concentration of resources and decisions on treatment intensifi-

cation. However, before widespread implementation, the model requires external validation on other patient cohorts.

Strengths and limitations of the study. The strengths of the study include the analysis of data over a long period (one year) covering various waves of the pandemic, the description of a specific organizational model and treatment protocols, as well as a direct comparison of outcomes for different GCs regimens and the development of a prognostic model. The main limitations are the retrospective design, the possible presence of unaccounted confounding factors, the potential incompleteness of data in electronic records, and the focus of outcome analysis primarily on patients transferred to the ICU (lack of detailed comparison with those who remained in the IOW). Also, the data on the number of patients in various analyses ($n = 950$, $N = 573$, $N = 644 + 306$) require clear interpretation and coordination.

Conclusion

The organization of IOW for high-flow oxygen therapy is a feasible strategy for managing patients with COVID-19-associated hypoxemic respiratory failure in conditions of a shortage of resuscitation beds, although a significant proportion (about 60%) of patients require escalation of respiratory therapy in the ICU.

The use of methylprednisolone at a dose of 1 mg/kg/day (bolus + prolonged infusion) in patients with severe COVID-19 transferred to the ICU was associated with lower mortality and a lower risk of requiring NIMV/IMV compared with the use of dexamethasone 20 mg/day in the study cohort.

The developed logistic regression model, which includes age, presence of diabetes mellitus, NEWS score, and type of GCS used, has good predictive ability for identifying patients in the ICU at high risk of requiring NIMV/IMV, and may be useful for clinical application after external validation.

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Artificial Intelligence Applications for Automatic Pain Assessment in the ICU (Short Review)

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Summary

Pain remains a major clinical challenge in the intensive care unit (ICU), especially in sedated, mechanically ventilated, or curarized patients due to their inability to self-report and the limited accuracy of behavioral tools. Therefore, innovative approaches must be developed. In this scenario, objective and observer-independent pain assessment can support and improve personalized analgesic management.

The aim of this review is to analyze the current artificial intelligence (AI) applications for automatic pain assessment (APA) in the ICU, focusing on the integration of biosignals, behavioral indicators, and multimodal data to detect nociceptive responses.

A systematic search was conducted in PubMed, Web of Science, and IEEE Xplore databases (2015–2025) using the terms pain assessment, critical care, artificial intelligence, machine learning, facial expression, pupillometry, heart rate variability, and nociception monitor. The scientific output was grouped into three main domains: behavioral and computer-vision methods, autonomic and electrophysiological indices, and multimodal and AI-driven integrated systems.

Conclusion. Although AI systems for APA in the ICU show promising performance, several challenges limit their clinical translation. Signal variability due to pharmacological, neurological, or hemodynamic factors may compromise model reliability. Moreover, the scarcity of labeled ICU datasets can affect generalizability. Ethical, regulatory, and interoperability issues should be addressed. Therefore, for routine implementation, large-scale validation across diverse ICU populations is required to confirm reliability, ensure fairness, and establish clinical utility.

Keywords: artificial intelligence; automatic pain assessment; nociception; intensive care unit; biosignals; multimodal monitoring; deep learning

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Introduction

Despite advances in critical care, pain is still highly prevalent in the intensive care unit (ICU), with an incidence of up to 70% in medical and surgical patients, at rest and mostly during procedures [1]. Untreated pain can induce cardiac instability, respiratory compromise, and immune suppression. Additionally, pain is often associated with agitation, delirium, and longer ICU stays [2], as well as chronic pain in ICU survivors [3].

Although the underlying reasons for this lack are complex, pain assessment is the main issue. In critically ill patients, proper pain evaluation remains challenging, particularly for those who are sedated, on mechanical ventilation, or unable to communicate, as only a limited number of reliable strategies are currently available [4]. In patients who can communicate, pain is evaluated through self-report. Meanwhile, in non-communicative ICU patients, behavioral observation tools like the Behavioral Pain Scale (BPS) and the Critical-Care Pain Observation Tool (CPOT) are commonly used [5]. They are recommended by clinical practice guidelines [6]. However, these tools tend to lack reliability in deeply sedated or paralyzed patients [7]. Additionally, their accuracy depends on proper training and active participation by the assessor, which increases the nursing workload. Furthermore, intermittent manual scoring cannot provide real-time, dynamic pain assessment, limiting its effectiveness in guiding prompt clinical decisions.

In this complex scenario, developing objective and automated methods to detect nociceptive responses and pain is essential. Recently, there has been increasing interest in using artificial intelligence (AI) and sensor technologies to achieve Automatic Pain Assessment (APA) in critical care [8,9]. This interdisciplinary field of study utilizes various data-driven techniques that can combine information from vital signs, facial expressions, and different biosignals to estimate nociception features and pain levels continuously [10].

On these premises, this review aims to provide a comprehensive, state-of-the-art overview of AI-based approaches for APA in the ICU. Specifically, the objective is to summarize current AI applications, evaluate their validation level and clinical applicability, and discuss future directions for integrating these technologies into routine critical care practice.

Materials and Methods

A literature search in PubMed, Web of Science, and IEEE Xplore (from 2015 to September 2025) was performed using combinations of: «pain assessment», «ICU» OR «critical care», «artificial intelligence», «machine learning», «nociception monitor», «pupillometry», «heart rate variability», «facial expression», «electrodermal activity», «multimodal pain». Peer-reviewed clinical studies in ICU settings and technical studies relevant to pain detection in the ICU were considered. Approximately 80 sources underwent full-text review; key findings are summarized by thematic category. Specifically, clinical applications of AI-based pain assessment are grouped into behavioral pain assessment and computer vision (CV) approaches, autonomic and electrophysiological pain modalities, and multimodal and AI-driven integrated systems.

Clinical Applications of AI-Based Pain Assessment

1. Behavioral pain assessment and computer-vision approaches. Conventional behavioral scales, including CPOT and BPS, remain the gold-standard bedside tools for non-communicative adult ICU patients and are guideline-endorsed [6]. AI-based strategies can be implemented for overcoming their limitations, such as observer dependence, intermittency, and reduced utility under deep sedation. For example, in the field of automated facial expression recognition, CV models can detect pain-related facial expression through facial action unit (AU) analysis. It is a framework originally developed within the Facial Action Coding System (FACS). Specifically, each AU corresponds to a specific muscle (or muscle group) contraction or facial movement (e.g., brow lowering, eye tightening, nose wrinkling), which can be objectively quantified from video data. Deep learning (DL)-based models, typically convolutional or recurrent neural networks, can automatically extract and classify these AUs to identify characteristic facial patterns associated with nociceptive responses [11, 12]. In a prospective ICU study, DL architectures fine-tuned and trained on video clips achieved approximately 80% accuracy for pain vs no-pain classification and generalized to unseen patients, with temporal video models outperforming single-frame models [13]. Nevertheless, ICU constraints such as tubes, masks, lighting, and sedation can limit the efficacy of these methods. Methodological attempts have been conducted to overcome these limitations. For example, a study by X. Yuan et al. [14] proposed a facial AUs-guided pain assessment network specifically designed to address one of the main challenges, which is facial occlusion due to tubes or masks. The DL model integrated an AU-guided module that automatically identified AUs in visible, non-occluded regions with a texture feature extraction module capturing both local and global facial patterns. These multimodal features were then fused in a pain assessment module to estimate pain state and intensity. Importantly, validated on both public (UNBC-McMaster shoulder pain datasets) and proprietary datasets, the approach outperformed conventional models in binary and multi-class pain classification as well as in intensity regression (accuracy > 90%).

Concerning other behaviors, body movement and ventilator synchrony may signal pain (or discomfort), but are less studied for automation in the ICU. Probably, multimodal systems may incorporate pose tracking and audio when applicable [9, 10].

2. Autonomic and electrophysiological pain modalities. Since physiological modalities can capture involuntary responses mediated by the autonomic nervous system (ANS), they can be adopted for developing APA systems [9, 10]. Among the most investigated approaches are pupillometry, heart rate variability (HRV), electrodermal activity (EDA), and multimodal nociception indices that integrate several biosignals through AI-driven algorithms. These methods offer the potential to continuously quantify nociceptive responses, reducing observer bias.

Infrared pupillometry quantifies the pupillary dilation reflex to noxious stimulation. Therefore, pupillometric indices such as the Pupillary Pain Index (PPI) may correlate with analgesic adequacy [15]. A recent prospective study evaluated the utility of videopupillometry for nociception assessment in deeply sedated, non-neurological ICU patients. The authors compared pupillary dilation reflex (PDR) responses to non-noxious versus noxious procedures, such as gentle shoulder touch versus endotracheal suctioning or repositioning. Results demonstrated that PDR significantly increased during noxious procedures, with a mean difference of approximately 32%. After adjusting for confounders, only the noxious procedure remained a significant predictor of pupil change. A PDR threshold of > 12% yielded 65% sensitivity and 94% specificity for nociception detection (AUC = 0.83) [16]. These findings support the approach as a promising and objective indicator of nociceptive responses in deeply sedated ICU patients. Nevertheless, neurologic injury and other limitations, such as lighting, can confound measurements.

Electrocardiogram (ECG)-derived parameters such as HRV were also investigated. It is the variation in time intervals between consecutive

heartbeats (the R–R intervals on an ECG), reflecting the balance and dynamic interaction between the sympathetic and parasympathetic branches of the ANS. This physiological marker of nociception was adopted to develop indices such as the Analgesia Nociception Index (ANI), which quantifies the autonomic response to pain or stress. The ANI index, ranging from 0 to 100, reflects parasympathetic predominance. In ICU patients, ANI showed moderate correlation with behavioral pain and a high negative predictive value for moderate-to-severe pain when ANI was high, although sensitivity/specificity were limited [17]. Importantly, arrhythmias, pacemakers, β-blockers, and non-pain stressors confound HRV-based indices.

Electrodermal activity (EDA) reflects changes in skin conductance mediated by sympathetic nervous system activation, which increases during stress, emotional arousal, or nociceptive stimulation [18–20]. When a painful or emotionally salient stimulus occurs, sweat gland activity rises, transiently decreasing skin resistance and generating measurable skin conductance responses (SCRs). In research and experimental settings, EDA has proven to be a reliable measure of pain intensity. Machine learning (ML) models trained on time- and frequency-domain SCR features, such as response amplitude, frequency, rise time, and recovery slope, have demonstrated good predictive performance for differentiating pain levels, as seen in datasets like BioVid or SenseEmotion [19]. However, several factors can affect the reliability of EDA in the ICU environment. Physiological conditions (e. g., hypoperfusion, neuropathies), pharmacological influences (e. g., vasopressors, sedatives, and anticholinergic drugs), and environmental factors (e. g., temperature, humidity, and electrode placement) can introduce artifacts or attenuate the signal.

Based on biosignals, multiparameter nociception monitors have been developed. The Nociception Level (NOL) integrates HR, HRV, pulse wave amplitude, skin conductance, and temperature into a composite 0–100 index using a proprietary (AI-derived) algorithm. In critical care, a randomized trial found NOL-guided analgesia reduced opioid dosing without worsening pain scores, with a trend toward shorter ICU stays [21, 22]. Moreover, in a prospective cohort study, T. S. Shahiri et al. [23] investigated the potential of NOL for pain assessment among mechanically ventilated patients able to self-report pain. Data was collected before, during, and after a non-nociceptive procedure (blood pressure cuff inflation) and a nociceptive procedure (endotracheal suctioning). Pain intensity (0–10 scale), CPOT scores, and NOL values were analyzed using non-parametric statistical tests. The authors found that NOL values significantly increased during endotracheal suctioning compared with pre- and post-procedure values and with cuff inflation ($P < 0.001$). NOL values also correlated with both self-reported pain and CPOT scores ($P < 0.05$). More recently, Bonvecchio et al. [24] conducted a retrospective cohort study to evaluate the same device in deeply sedated and curarized critically ill patients, a population in which traditional pain monitoring is unreliable. The study compared NOL performance with standard neurovegetative indicators (e. g., heart rate, blood pressure) and examined its relationship with sedation depth measured by bispectral index (BIS). Results showed that NOL demonstrated superior accuracy in detecting nociceptive events across the overall cohort compared with conventional physiological indicators. In non-curarized patients, all indices, including NOL, identified nociceptive stimulation, whereas in paralyzed patients, only NOL reliably detected nociceptive responses. Since electroencephalography (EEG)-derived sedation indices are not able to capture nociception, in the curarized group, no correlation was found between BIS values and NOL. On the other hand, sensor complexity, artifact susceptibility (e. g., movements), hemodynamic instability due to shock, and arrhythmias are key limitations.

3. Multimodal and AI-driven integrated systems. Combining different modalities such as facial action units, biosignals, and clinical data can offer more robust pain detection. For example, Othman et al. [25] fused facial analysis with EDA and used three different approaches, including random forest (RF), Long-Short Term Memory Network (LSTM), and LSTM with the sample-weighting method (LSTM-SW). They found that models improved multi-level pain classification over single-modality models, supporting complementary information across channels. Moreover, classical ML, such as support vector machines (SVMs) and RFs, and DL architectures have been applied.

A particularly advanced framework for ICU pain evaluation is represented by the Intelligent Intensive Care Unit (I2CU) system [26]. This architecture integrated multimodal sensing and DL to enable continuous, real-time visual and physiological assessment of patients' states, including pain, acuity, mobility, and delirium risk. The system collected data simultaneously from multiple modalities such as imaging, accelerometry, electromyography (EMG), environmental sensors (light and noise), and vital signs. For pain assessment, the visual module applied an AUs detection pipeline based on the FACS, using a multitask cascaded convolutional network (MTCNN) and a Swin Transformer model trained to recognize twelve AUs that align with established behavioral pain metrics such as CPOT and BPS. Moreover, cameras captured posture and mobility data analyzed with CV-based You Only Like Once (YOLO) models, while physiological time-

Table. AI-based tools and methods for automatic pain assessment in ICU patients.

Method	Input	AI technique	Validation level (Internal/External)*	Key limitations	Ref.
Facial expression analysis	Face video	DL (CNN, RNN)	ICU (80% accuracy) (Internal validation)	Occlusion, sedation effects, lighting/ethnic variability	[13]
AU-guided facial pain network	Face video + texture features	DL (CNN + TFE module + fusion)	External validation on public dataset (UNBC-McMaster) and internal ICU dataset (>90% accuracy)	Occlusion handling, need for large annotated datasets	[14]
PPI	Pupil reflex	Algorithmic index	External validation in OR setting; internal discriminative study in the ICU (AUC 0.83)	Drug/neurologic/lighting confounders; stimulus required	[15–16]
HRV (ANI)	ECG/PPG HRV	Proprietary index	ICU diagnostic study; moderate performance, high NPV (Internal validation)	Arrhythmias/pacing/ β-blockers; low specificity	[17]
Skin conductance (EDA)	EDA (SCRs)	Feature engineering + ML (SVM, RF, CNN)	Experimental datasets (e.g., BioVid, SenseEmotion) (External validation)	Motion/temp confounders; peripheral shutdown	[19]
Multiparameter (NOL)	HR/HRV, PPG, EDA, temp	Composite ML index	OR validated (External validation); ICU RCT opioids, similar pain scores (Internal validation)	Artifacts; sensor complexity; hemodynamic instability	[21–24]
Vital-sign ML model	HR, BP, RR, age, sex, RASS	ML (RF model)	Retrospective ICU (>10,000 pts; AUC 0.903) (Internal validation)	Retrospective bias; limited external validation	[29]
Multimodal fusion (EDA + video)	Video + EDA	DL (CNN/LSTM/ transformers)	Internal prototype validation with cross-modal fusion; external validation pending	Data needs; integration and missing-data handling	[25]
I2CU (Intelligent ICU)	Video, depth, EMG, vitals, environment	DL (FACS-based AU + Swin Transformer + YOLO + transformers)	Internal real-ICU pilot validation; external multicenter validation pending	Complex infrastructure; computational load; data privacy issues	[26]

Note. * Internal validation refers to testing within the same dataset or cohort, while external validation refers to independent datasets or different clinical contexts. DL — Deep Learning; CNN — Convolutional Neural Network; RNN — Recurrent Neural Network; AU — Action Unit; TFE — Texture Feature Extraction; HRV — Heart Rate Variability; HR — Heart Rate; PPI — Pupillary Pain Index; BP — Blood Pressure; EMG — Electromyography; ECG — Electrocardiogram; PPG — Photoplethysmography; EDA — Electrodermal Activity; SCR — Skin Conductance Response; OR — Operating Room; ICU — Intensive Care Unit; ANI — Analgesia Nociception Index; NOL — Nociception Level Index; SVM — Support Vector Machine; RF — Random Forest; RASS — Richmond Agitation–Sedation Scale; YOLO — You Only Look Once; LSTM — Long Short-Term Memory network.

series were processed using self-supervised transformers trained on more than 300 clinical variables to estimate acuity and autonomic states. The AI system merged these heterogeneous data streams through a real-time ML engine and displayed them via a clinician dashboard showing inferred pain scores, mobility indices, delirium risk, and environmental parameters. Overall, the I2CU architecture demonstrated how AI-enhanced multimodal sensing and FACS-based facial analysis can operationalize continuous and interpretable pain assessment in real ICU environments, addressing many of the current barriers to observer-independent, real-time nociception monitoring.

Although these approaches are promising, for generalizability, larger and diverse datasets, and standardized evaluation are mandatory [8, 9].

The characteristics of AI-based tools and methods for automatic pain assessment in ICU patients are presented in the table.

Limitations and Challenges

Several critical issues influence the effective implementation of AI strategies for APA in the ICU. First, there is the fundamental distinction between pain and nociception. In particular, current monitoring systems can capture only physiological responses, not the patient's subjective experience of suffering [10]. Second, patient variability and confounding factors, such as arrhythmias, pharmacologic agents, neurologic injury, or vasopressor use, can significantly alter the biosignals on which AI algorithms rely, thereby affecting model reliability [20–24]. A third challenge is the limited availability of labeled ICU datasets suitable for model training. Because pain cannot be objectively measured, most datasets use proxy labels derived from behavioral tools like CPOT or BPS, which introduce noise and variability into AI training [8, 9]. From an operational standpoint, real-time integration into ICU workflows raises additional concerns, such as system interoperability. Moreover, clinician acceptance and training remain essential as AI outputs must be interpretable and easily understood by healthcare professionals to foster trust and effective use at the bedside [8]. Finally, there are important regulatory, ethical, and fairness considerations. These include safeguarding data privacy, ensuring unbiased model performance across diverse patient populations, and maintaining human oversight in decision-making processes through human-in-the-loop designs [8, 9].

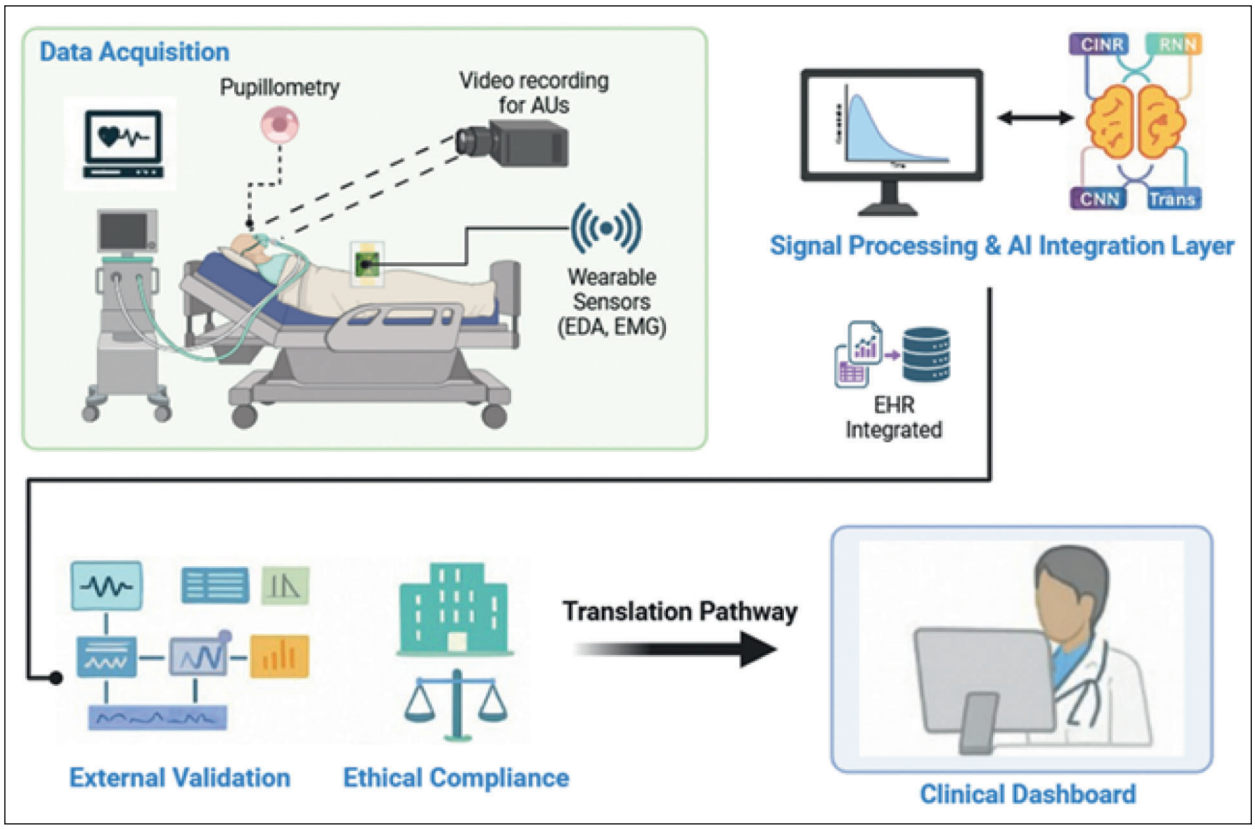

Fig. Schematic overview of AI-driven automatic pain assessment in the ICU (Created by Marco Cascella).
Note. Physiological, behavioral, and multimodal data are collected through bedside sensors and processed by deep learning models to detect nociceptive responses. After external validation and following ethical compliance, model outputs are integrated into clinical dashboards for real-time, observer-independent pain monitoring and personalized analgesic management. AUs — action units; EDA — electrodermal activity; EMG — electromyography; EHR — electronic health record.

Perspectives and Future Directions

Near-term deployment will likely be as decision-support: continuous indices that prompt reassessment or preemptive analgesia, with electronic health record (EHR) integration, trend displays, and smart alarms [21]. Goal-directed analgesia guided by objective indices, for instance, NOL or ANI targets, may reduce drug exposure without compromising comfort [27]. Interestingly, multimodal strategies could integrate brain activity. For example, Chen et al. [28] developed a pain detection framework based on EEG signals and deep convolutional neural networks to distinguish induced pain from resting states.

The multimodal integration may also include clinical data derived from validated assessment tools (Fig.).

A recent large-scale study explored the use of ML applied to vital sign data for automatic pain visualization in ICU patients. The authors investigated whether incorporating individual baseline deviations in physiological parameters could enhance model accuracy. Using data from over 10,000 adult ICU patients and more than 117,000 CPOT assessments, a random forest model was trained with arterial pressure, heart rate, respiratory rate, age, gender, and sedation score as predictors. Pain probability was defined as the likelihood of a CPOT $\geqslant 3$, adjusted for each patient's baseline. The model achieved excellent discriminative performance (AUC = 0.903), demonstrating that the use of different clinical and instrumental inputs can enhance the sensitivity of the AI-based automatic pain detection and, ultimately, develop reliable and scalable systems [29].

Nevertheless, another aspect that needs to be clarified is the definition of integration pathways among the different study components, such as between various biosignals and between biosignals and behavioral approaches. For example, a recent observational study compared pupillary response and skin conductance for nociception assessment in unconscious or deeply sedated ICU patients. Fifty-one adults with acute brain injury or under deep sedation underwent tetanic stimulation while both parameters were recorded simultaneously. Pupillary dilation was quantified using the PPI, whereas skin conductance was expressed as the number of peaks per second. Results showed that more than half of the patients (55%) had insufficient analgesia according to the PPI, whereas only 29% displayed measurable SC activity. Importantly, no significant correlation was found between EDA-derived indices and pupillary responses [30]. Therefore, careful integration of differ-

ent modalities is warranted to improve model robustness and reduce false interpretations.

Other challenges need careful attention. Advances in sensing technology (wearables, non-contact vitals) and AI (federated learning, explainable models, multimodal fusion) will enhance the robustness and acceptance of the developed technology [8]. Due to the varied methods used in AI research, interdisciplinary standards for datasets, benchmarks, and reporting are essential [8]. Ethics must also be considered. For instance, adoption requires staff training and transparent communication with patients, as well as ensuring data privacy, reducing algorithmic bias, and maintaining human oversight in decision-making to preserve clinical accountability and patient trust [31]. Ultimately, APA could turn pain into a real «continuous vital sign,» particularly for patients unable to speak for themselves.

Conclusion

To date, research suggests that AI-enabled APA can augment bedside care by continuously detecting nociceptive responses when traditional behavioral scales are infeasible. Evidence supports the utility of pupillometry, HRV-based indices, and multiparameter monitors such as NOL. Furthermore, while facial analysis and multimodal AI systems are promising, these approaches require larger, diverse validation. Further high-quality investigations are needed for the thoughtful integration of APA methods in ICU care pathways.

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ERRATUM
***Obshchaya Reanimatologiya* = *General Reanimatology*. 2020; 16 (6): 34**

Erratum was claimed by authors. The authors pointed out a misprint in the Materials and Methods section [In Engl.] on p. 34.

Correction to the article: «Use of Sorbent Based on Hypercrosslinked Styrene-Divinylbenzene Copolymer With Immobilized LPS-Selective Ligand in Hemoperfusion for Treatment of Patients With Septic Shock».

Marat A. Magomedov, Timur G. Kim, Sergei V. Masolitin, Artur V. Yaralian, Evgeny Yu. Kalinin, Vladimir M. Pisarev. https://doi.org/10.15360/1813-9779-2020-6-31-53.

«…who were admitted to the intensive care unit (ICU) in 2020». should be replaced with «... who were admitted to the intensive care unit (ICU) in 2019 and 2020».

The correct option [In Engl.] is:

«The study included 9 surgical patients with clinical signs of septic shock (SEPSIS-3, 2016) who were admitted to the intensive care unit (ICU) in 2019 and 2020».

ERRATUM
***Общая реаниматология*. 2020; 16 (6): 35**

Erratum заявлен авторами. Авторы указали на опечатку в разделе «Материалы и методы» на с. 35.

Исправление к статье: «Использование сорбента на основе сверхсшитого стирол-дивинилбензольного сополимера с иммобилизованным ЛПС-селективным лигандом при гемоперфузии для лечения пациентов с септическим шоком».

М. А. Магомедов, Т. Г. Ким, С. В. Масолитин, А. В. Яралян, Е. Ю. Калинин, В. М. Писарев. https://doi.org/10.15360/1813-9779-2020-6-31-53

«… поступивших в отделение реаниматологии и интенсивного лечения (ОРИТ) в 2020 г.» необходимо заменить на «… поступивших в отделение реаниматологии и интенсивного лечения (ОРИТ) в 2019 и 2020 гг.»

Правильный вариант:

«В исследование включили 9 пациентов хирургического профиля с клиническими признаками септического шока (СЕПСИС-3, 2016), поступивших в отделение реаниматологии и интенсивного лечения (ОРИТ) в 2019 и 2020 гг.»

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