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ERRATUM 75 **ОПЕЧАТКИ**

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Predictors of Complications Related to Cardiac Ablation for Atrial Arrhythmias

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Summary

The heterogeneity of the patient population and the lack of uniform approaches to periprocedural management highlight the importance of investigating the predictors of catheter ablation (CA) related complications in patients with atrial arrhythmias.

Aim of the study: to identify risk factors for procedure-related (PR) and procedural sedation and analgesia (PSA)-related complications in patients with atrial arrhythmias.

Materials and Methods. A single-center retrospective cohort observational study analyzed 2,340 electronic medical records (EMRs) from the I. I. Mechnikov NWSMU database from 2015 to 2022. A total of 1,793 EMRs were included in the study. All the patients underwent radiofrequency CA for atrial arrhythmia under procedural sedation and analgesia. The risk factors for PR- and PSA-related complications were identified using single-factor regression analysis and multivariate logistic regression with Jamovi 2.3.21 and IBM SPSS Statistics 26 software.

Results. The PR and PSA-related complication rates were 3.29% and 0.73%, respectively. Hemopericardium/cardiac tamponade with an incidence of 1.45% and cerebral stroke/TIA documented in 1.17% of cases predominated among the PR complications. PSA-related complications included postoperative nausea and vomiting syndrome (0.22 %) and respiratory depression (requiring mechanical ventilation in 0.06% and non-invasive ventilation in 0.45%). Of all PR complications, 30.5% were documented in patients aged 70–74 years. BMI > 30.0 kg/m² (adjusted OR, 1.963; 95% CI, 1.09–3.36; p=0.023), age > 69 years (adjusted OR, 3.081; 95% CI, 1.764–5.383; P<0.001), pain severity on the numerical rating scale (NRS) > 3 points (adjusted OR, 4.317; 95% CI, 2.390–7.800; P<0.001), and previous CA procedure in the patient's history (adjusted OR, 10.276; 95% CI, 4.006–26.354; P<0.001) were found to be risk factors for the development of PR complications, whereas BMI > 35 kg/m² (adjusted OR, 4.955; 95% CI, 1.485-16.535; P=0.009) and duration of CA procedure > 142 min (adjusted OR, 11.070; 95% CI, 2.440-50.228; P=0.002) were found to be risk factors of PSA complications.

Conclusion. The following independent predictors of CA-related complications were identified: patient-related factors such as BMI > 30.0 kg/m² and age > 69 years, as well as procedure-related factors such as duration of CA > 142 min, history of CA, and pain intensity > 3 NRS points.

Keywords: procedural complications; catheter ablation; risk factors; atrial arrhythmias; procedural sedation and analgesia

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

Introduction

According to projections for the period 2030–2034, the global incidence of atrial fibrillation (AF) in men will be 16.08 million, with 1.01 million disease-related deaths, while the global incidence in women will be 16.85 million, with 1.49 million deaths [1]. Atrial fibrillation (AF) is a progressive and multifactorial arrhythmia often associated with the most common cardiovascular diseases. These conditions share traditional cardiovascular risk factors such as hypertension, high body mass index (BMI), alcohol consumption, smoking, and a high sodium diet [1]. Catheter ablation (CA) procedures for atrial fibrillation are becoming increasingly common. Because CA alters the pathogenetic mechanism underlying the onset and persistence of AF, early intervention can limit disease progression and improve clinical outcomes [2].

Over the past decade, technological advances in CA for AF have aimed to shorten the ablation procedure and improve its safety [3]. Complications such as cardiac tamponade, stroke, pulmonary vein stenosis, vascular access-related sequelae (e. g., bleeding, hematoma, femoral pseudoaneurysm), and pneumothorax occur rapidly and can be fatal [4].

Identifying patients at high risk for complications and considering predictors of their development in preprocedural planning remains a priority [5]. Increasing age is independently and significantly associated with the total number of complications [5]; however, low complication rates and favorable outcomes after CA have been reported even in patients

with AF aged ⩾80 years [6]. Data on the relationship between complications and patient sex have been equivocal, but the study by R. Yadav et al. demonstrated the absence of sex differences in the safety and efficacy of ablation [7]. Studies in recent years did not show any association between BMI and complications of CA for AF [8,9], but procedure time and radiation exposure were increased in obese patients [10].

During the complex and prolonged procedure of CA for AF, patients often experience excruciating pain when the ablation reaches the autonomic nerve distribution area or the esophageal region [11]. Sedation and analgesia are necessary to reduce pain and maintain catheter stability. General anesthesia increases patient comfort during the procedure and ensures safety of transseptal puncture and accuracy of catheter manipulation [12]. However, general anesthesia is associated with increased total procedure time and potential complications such as aspiration, anaphylaxis, and trauma associated with tracheal intubation.

A study of 300 patients comparing the use of procedural sedation and analgesia (PSA) [13] with general anesthesia in patients with atrial fibrillation showed no significant difference in complication rates between the groups. A higher American Society of Anesthesiologists (ASA) anesthesia risk was found with general anesthesia (45% vs. 75%, P<0.01), and procedure time was shorter in patients with PSA (110 vs. 139 min, P<0.001) [14]. Although general anesthesia is the standard in some centers, CA procedures can also be performed under PSA using propofol as the only anesthetic and fentanyl for analgesia [12].

Between 2010 and 2019, the number of CAs for AF performed under general anesthesia (36.1–40.5%; P=0.02) and in deep sedation (22.7–27.5%; P<0.01) increased, while the frequency of PSA with a Richmond Agitation-Sedation Scale (RASS) score of –1 to –2 decreased to 9.2%. Nevertheless, in 2019, 32.0% of CA for AF were performed with PSA [15].

Multivariate analysis showed that each five-year increase in age, female sex, and ASA > III were associated with a 7.0% (P<0.0001), 9.0% (P=0.032), and 200.0% (P<0.0001) increase in the incidence of PSA with RASS –1/–2 scores, respectively [4]. A 2019 meta-analysis including 9 observational studies of CA for AF compared general anesthesia and PSA. General anesthesia/deep sedation was associated with a reduced risk of AF recurrence (OR: 0.79, 95% CI 0.56 to 1.13, P=0.20) and complications (OR: 0.95, 95% CI 0.64 to 1.42, P=0.82), although the differences were not statistically significant [16]. According to Y. Yokokawa et al., who compared the efficacy, safety, clinical outcomes and costs of CA for AF performed with PSA and general anesthesia, the prevalence of procedural complications (PC) was similar in the two groups (4% vs. 4%, P=0.89). General anesthesia was associated with a small (~7%) increase in total cost due to longer observation time in the recovery room [17]. Patient demographics, comorbidities, and differences between centers and anesthesia techniques used were predictors of complications. There is an urgent need to identify modifiable risk factors for complications of CA of atrial arrhythmias under PSA.

The aim of this study was to determine risk factors for the development of PC and PSA complications in patients undergoing CA of atrial arrhythmias under PSA.

Materials and Methods

A single-center retrospective cohort observational study was approved by the Local Ethical Committee (LEC) of the I. I. Mechnikov Northwestern State Medical University (I. I. Mechnikov NWSMU), protocol No. 6 of the LEC meeting dated 14.06.2023. We conducted consecutive screening of 2340 electronic medical records (EMR) from the database of I.I. Mechnikov NWSMU for the period from 03.03.2015 to 14.07.2022.

Inclusion and exclusion criteria are shown in the study scheme (Fig. 1).

CA for AF and antiarrhythmic therapy were performed according to the 2014 and 2019 updates of the American College of Cardiology/American Heart Association/Heart Rhythm Society (AHA/ACC/HRS) guidelines. [18,19]. Radiofrequency CA was routinely performed in the radiosurgical operating room using PSA while monitoring RASS scores (–1 to –3). PSA was induced by intravenous fractional bolus injection of diazepam, propofol, and fentanyl (Table 1). Monitoring during surgery was performed with a four-lead body surface electrocardiogram and intracardiac electrograms (CARTO® 3 device, Biosense Webster, Johnson & Johnson MedTech, USA), measurement of HR, SpO_2, and NIBP (GE B 30, General Electric Company, USA).

To avoid hypoxemia, oxygen therapy was administered, starting in most cases with a flow rate of 2 L/min (or 1 L/min in patients with chronic obstructive pulmonary disease) through a nasal cannula. The flow rate was increased when SpO_2 decreased. The ablation index was considered during CA. In patients with RASS –1 to –2, pain was assessed by verbal contact during CA using the NRS.

The following data were collected in the study: sex, weight, height, age, ASA score [20], Charlson Comorbidity Index (CCI) score [21], CHA_2DS_2-VASc risk score (Congestive heart failure, Hypertension, Age ⩾75 years, Diabetes mellitus, Prior Stroke or TIA or Thromboembolism) [22] and HAS-BLED (Hypertension, Abnormal renal-liver function, Stroke,

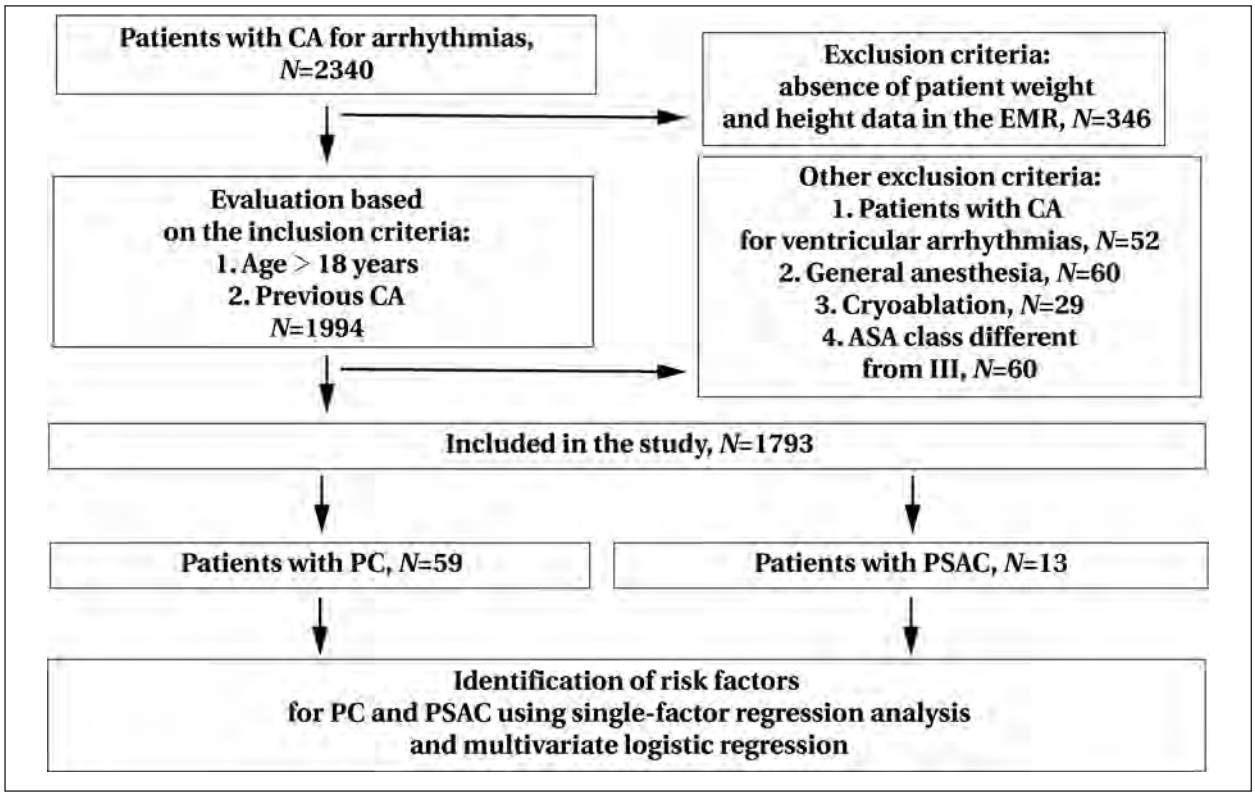

Fig. 1. Study design.
Note. PC, procedural complications; PSAC, «procedural» sedation and analgesia complications.

Bleeding history or predisposition, Labile international normalized ratio, Elderly (65 years), Drugs or alcohol concomitantly) [23], medications, need for surgery, previous procedures, need for electrical cardioversion, duration of procedure, anesthesia, level of sedation according to RASS [24], doses of hypnotics and analgesics, frequency and pattern of PC, complications of PSA including pain score using the Numerical Rating Scale (NRS) [25], length of hospital stay.

Statistical analysis was performed using Jamovi 2.3.21 and IBM SPSS Statistics 26 software packages. Data were reported as mean and standard deviation *(M±SD)* or median and interquartile range of *Me (Q1; Q3)*, depending on the distribution. Qualitative variables were reported as absolute numbers *(N)* and percentages (%). Normality of the distribution of quantitative variables was determined by the Shapiro–Wilk test. In the comparative analysis of 2 independent groups, the Mann-Whitney test was used. The influence of parameters on target binary variables was evaluated using Pearson's χ^2 test. The Bonferroni correction was used for multiple comparisons. For multivariate analysis, we selected factors that showed a significant effect on the outcome. From these factors, independent predictors were selected by binary logistic regression (by sequential elimination using the Wald statistic), and adjusted odds ratios *(ORs)* were calculated. Cutoff points for quantitative parameters were determined by ROC curve analysis. Binary logistic regression was used to determine ORs and adjusted ORs *(AORs)*. Differences were considered significant when $P<0.05$. Risk factors for the development of PC and PSA complications were identified using single-factor regression analysis and multivariate logistic regression.

Results and Discussion

The EMR data of 1793 patients with CA for atrial arrhythmias under PSA were included in the study. The study design is shown in Fig. 1.

Patient characteristics and interventions performed are shown in Table 1.

In our study, a low incidence of PC (3.29%) and 0.05% in-hospital mortality was observed, which is in agreement with the data of Y. Yokoyama, et al. (complication rate 3.4% and in-hospital mortality 0.04%) [5]. All complications were detected during the intraoperative or early postoperative period. In a meta-analysis by Jafry et al, the authors also found no significant difference in complication rates between groups of patients discharged on the day of surgery or later [26]. They showed that vascular/hemorrhagic complications such as hemopericardium/tamponade (1.45%) and neurological complications such as acute cerebrovascular accident/transient ischemic attack (1.17%) were the most common PC, which is in line with results from other centers [27]. In our study, no atrioe-

Table 1. Patient and intervention characteristics.

Parameters	Values (*N*=1793)
Age, years *(M±SD)*	58.7±12.4
Age groups, years, *N* (%)	
under 60	829 (46.2)
60–64	307 (17.1)
65–69	340 (19.0)
70–74	211 (11.8)
75–79	78 (4.4)
80–84	23 (1.3)
85 and older	5 (0.3)
Female sex, *N* (%)	905 (50.5)
Weight, kg *(Me (Q1; Q3))*	78.0 (69.0; 90.0)
Height, cm *(Me (Q1; Q3))*	170.0 (164.0; 177.0)
BMI, kg/m² *(Me (Q1; Q3))*	26.4 (23.7; 29.9)
BMI, kg/m², WHO classification, *N* (%)	
18.5–25.0 normal	639 (35.6)
25.0–30.0 overweight	692 (38.6)
30.0–35.0 obesity I	308 (17.2)
35.0–40.0 obesity II	104 (5.8)
>40,0 morbid obesity	35 (2.0)
16–18.5 weight deficit	15 (0.8)
Comorbidities, *N* (%)	
Hypertension	320 (17.8)
CHD	132 (7.3)
NYHA class I heart failure	6 (0.3)
NYHA class II heart failure	16 (0.9)
NYHA class III heart failure	1 (0.1)
History of ACVA	15 (0.8)
Diabetes mellitus	62 (3.5)
Score, points *(Me (Q1; Q3))*	
CCI	2 (1; 3)
CHA_2DS_2-VASc	1 (0; 1)
HAS-BLED	0 (0; 1)
The use of medications, *N* (%)	
Amiodarone	217 (12.1)
β-blockers (bisoprolol)	1793 (100)
Procedures	
RF pulmonary vein isolation, *N* (%)	1552 (86.6)
RFA of the cavo-tricuspid isthmus, *N* (%)	61 (3.4)
RF AV node modification, *N* (%)	132 (7.4)
RFA of arrhythmogenic substrate for atrial extrasystoles, *N* (%)	43 (2.4)
Duration of procedure, min *(Me (Q1; Q3))*	120.0 (70.0; 155.0)
Electrical cardioversion during procedure, *N* (%)	593 (33.1)
History of CA, *N* (%)	32 (1.8)
Average length of hospital stay, days *(Me (Q1; Q3))*	5 (3; 7)
PSA characteristics	
Frequency of RASS –1 to –2 sedation, *N* (%)	1188 (66.3)
Dose of propofol, mg/kg *(Me (Q1; Q3))*	1.613 (1.295; 2.439)
Dose of diazepam, mg/kg *(Me (Q1; Q3))*, *N*=66	0.131 (0.120; 0.166)
Dose of fentanyl, μg/kg *(Me (Q1; Q3))*	1.724 (1.351; 3.333)
Frequency of NRS >3, *N* (%)	182 (15.3%)
Frequency of RASS – 2 to – 3 sedation, *N* (%)	605 (33.7)
Dose of propofol, mg/kg *(Me (Q1; Q3))*	2.439 (2.151; 2.857)
Dose of diazepam, mg/kg *(Me (Q1; Q3))*, *N*=39	0.143 (0.125; 0.165)
Dose of fentanyl, μg/kg *(Me (Q1; Q3))*	2.3353 (1.961; 2.857)

Note. Samples of patients who differed in level of sedation according to the RASS scale and accordingly had different doses of hypnotics and analgesics are shown. For Tables 1, 3–6: CHD, coronary heart disease; ACVA, acute cerebrovascular accident; NYHA, New York Heart Association; CCI, Charlson Comorbidity Index; CHA2DS2-VASc, Congestive heart failure (Hypertension, Age ⩾75 years, Diabetes mellitus, Prior Stroke or TIA or Thromboembolism); HAS-BLED — Hypertension, Abnormal renal-liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly (65 years), Drugs or alcohol concomitantly; RF, radiofrequency; RFA, radiofrequency ablation; AV, atrioventricular.

sophageal fistula formation or phrenic nerve injury was found. Complicated PSA occurred in 0.73% of cases, which was significantly lower than in the study by Y. Yokokawa et al. (4.0%) [17] and R. Garcia et al. (2.9%) [15]. PSA complications were represented by postoperative nausea and vomiting (PONV) syndrome in 4 (0.22%) patients, respiratory depression related to anesthetic effects requiring ventilatory support in 1 (0.06%) patient, and NIVL in 8 (0.45%) patients. Periprocedural complications are shown in Table 2.

We found that patients with PC were significantly older than those without (Table 3), which is consistent with the literature [4].

In the 70–74 year age group, PC was detected in 30.5% (18/59) of patients, while 11.1% (193/1734) of patients did not have PC, *P*<0.001 (Table 3). Patients aged 70–74 years were 3.5 times more likely to develop PC (OR: 3.51; 95% CI: 1.97; 6.22, *P*<0.001). In contrast to the study by Y. Y. Liu et al., in our study, AF patients aged ⩾80 years did not differ in the number of PC identified [6].

R. Yadav et al. showed no effect of sex on safety and efficacy of ablation [7]. In our study, women had significantly more PC detected, P=0.030. Similar data were obtained by D. D. Spragg et al. [28] and M. L. Campbell et al. [29] who showed that the incidence of PC was significantly higher in women. Ac-

Table 2. Periprocedural complications in the studied patients (*N*=1793).

Complication	Frequency, *N* (%)
Procedural	
Hemopericardium/tamponade	26 (1.45)
Acute myocardial infarction	1 (0.06)
AV block	3 (0.17)
Conduction blocks and arrhythmias	1 (0.06)
Hemothorax	2 (0.12)
ACVA/TIA	21 (1.17)
Hematoma in the vascular access area	6 (0.33)
Intraoperative blood loss requiring blood transfusion	5 (0.28)
Total	59 (3.29)
Frequency of complications during previous CA	7 (0.39)
Procedural sedation and analgesia related	
PONV	4 (0.22)
Need for non-invasive lung ventilation after the procedure	8 (0.45)
Need for lung ventilation after the procedure	1 (0.06)
Total	13 (0.73)

Note. TIA, transient ischemic attack; AV, atrioventricular; PONV, postoperative nausea and vomiting.

cording to M. L. Campbell et al., neurological complications such as stroke/TIA were found in 0.51% of women and 0.39% of men, and intraprocedural mortality was 0.25% in women and 0.19% in men [29].

According to the data of a research team, there was no relationship between BMI and PC in relation to CA for AF [9]. Similar data were obtained by R. Providência et al., but two patients with high BMI had atrio-esophageal fistula and one patient with morbid obesity developed acute left ventricular failure during ablation [8]. In the work of S. D'Souza et al., obesity was associated with an increased risk of vascular/hemorrhagic complications [30]. Analysis of our data showed that morbid obesity was associated with the development of PC, $P<0.001$.

In our study, coronary heart disease (CHD) was found in 16.9% of patients with PC and 7% without PC ($P=0.004$, Table 3), which is consistent with the data of G. Steinbeck et al. (18.6% of patients with PC) [31].

Significant differences in CHA_2DS_2-VASc ($P=0.029$) and HAS-BLED ($P<0.001$) scores were observed between patients with and without PC (Table 3). CHA_2DS_2-VAS score $\geqslant 1$ was observed in the group with PC, similar to the study by E. Yang et al. [32]. As shown by K. Senoo et al., the HAS-BLED score was significantly associated with the risk of bleeding (any clinically significant bleeding: OR: 1.85; 95% CI: 1.43–2.40, $P<0.001$; major bleeding: OR: 2.40; 95% CI: 1.28–4.52; $P=0.007$) [33]. In our study, a HAS-BLED score $\geqslant 1$ point was associated with a 2.3-fold increased risk of developing PC (OR: 2.364; 95% CI; 1.404–3.981, $P=0.001$).

A history of previous ablation significantly increased the incidence of PC, $P<0.001$ (Table 3), similar findings were reported by Szegedi et al [34].

Procedural complications were found to increase the length of hospital stay by >7 days (Table 3), in contrast to the data obtained by A. Gupta et al [35].

The level of peri- and post-interventional pain experienced is considered a determinant of patient satisfaction with the ablation procedure [36]. In PO, a pain score of >3 points was recorded more frequently in patients with PSA with a RASS level of –1 to –2, $P<0.001$, and the fentanyl dose was higher in the PC group, $P=0.001$. The studied parameters in patients with and without PC are shown in Table 3.

Longer procedure times were associated with a higher incidence of PSAC ($P<0.001$) (Table 3).

B. Cronin et al. showed that minimal to moderate sedation during cryoablation is effective in most patients with AF, whereas deep sedation or general anesthesia is mandatory for RFA with 3D electroanatomic mapping, as the success of the procedure depends on minimal patient movement [37, 38]. Sedation is a continuum with a wide range of levels of consciousness, and the transition to deeper levels can be rapid and not always predictable [39].

In our study of 1793 patients, respiratory depression requiring ventilatory support occurred in 1 (0.06%) patient (RASS sedation level –2 to –3; propofol dose 2.439 (2.151; 2.857) mg/kg and fentanyl dose 2.3353 (1. 961; 2.857) μg/kg), and NIVL was performed in 8 (0.45%) patients (of whom 5 had RASS sedation level –1 to –2; propofol dose 1.613 (1.295; 2.439) mg/kg and fentanyl dose 1.724 (1.351; 3.333) μg/kg) (Tables 1, 2).

In a study of drug-related complications in a cohort of 3211 patients with AF undergoing deep sedation during CA [40], one patient (0.03%) required ventilatory support and 47 (1.5%) required NIVL. The mean doses of propofol, midazolam, and fentanyl were 33.7±16.7 mg, 3.0±11.1 mg, and 0.16±2.2 mg, respectively. Norepinephrine was administered to 396 of 3211 patients (12.3%) for hypotension (mean arterial pressure <60 mmHg). No hypotensive patients requiring vasopressor support were observed in our study.

In our study, 4 (80.0%) of 5 patients with PSA complications and a RASS score of >3 points received fentanyl 7.0 μg/kg at a sedation level corresponding to a RASS score of –1 to –2 points (Table 4). According to the literature, the use of higher doses of opioids is associated with a higher risk of adverse effects, morbidity, longer recovery time, and higher costs [41]. The length of hospital stay was not significantly different between the study groups (Table 4).

Multivariate analysis by J. Plášek et al. showed that older age was an independent predictor of major vascular complications in men [42]. The multivariate analysis showed that age >69 years was a predictor of PC, increasing their risk 3.08-fold (Table 5), in contrast to the study by A. Numminen et al. which found that age and weight were not significant predictors of PC [43].

Age $\geqslant$65 years ($P=0.0231$), female sex ($P=0.0438$), hypertension ($P=0.0488$), CHA_2DS_2-VASc score $\geqslant 2$ ($P=0.0156$) and previous CA for AF in the study by N. Szegedi et al. were associated with the development of complications in a single factor analysis [34]. Previous CA for AF does not rule out the possibility of posterior wall thinning, atrial septal changes, and adhesions in the vascular access area, which may explain the technical difficulties in performing CA [44, 45]. Furthermore, repeated CA procedures increase the risk of developing pulmonary vein stenosis [46]. Multivariate analysis by N. Szegedi et al. showed that the only independent predictor of AF was a history of previous AF ablation (AOR: 3.18; 95% CI 1.99–5.08; $P<0.0001$) [34]. In multivariate analysis, we found similar results: previous CA increased the odds of PC by 10.2-fold compared to patients without previous CA (Table 5).

According to the US National Registry 2005–2013, obesity was an independent predictor of PC (AOR: 1.39; 95% CI: 1.20–1.62) and was asso-

Table 3. Comparison of patients with atrial arrhythmias with or without procedural complications of CA.

Parameter	Values in patients		*P*-value
	without PC, *N*=1734	with PC, *N*=59	
Age, years *(M±SD, Me (Q1; Q3))*	58.5±12.4	64.6±10.6	<0.001
	60.5 (52.0; 67.0)	68.0 (59.0; 73.0)	
Age groups, years (*N* (%))			
Under 60	814 (46.9)	15 (25.4)	0.001
60–64	297 (17.1)	10 (16.9)	0.968
65–69	329 (19.0)	11 (18.6)	0.924
70–74	193 (11.1)	18 (30.5)	<0.001
75–79	75 (4.3)	3 (5.1)	0.767
80–84	22 (1.3)	1 (1.7)	0.791
85 and older	4 (0.2)	1 (1.7)	—
Female sex, *N* (%)	867 (50.0)	38 (64.4)	0.030
Weight, kg *(Me (Q1; Q3))*	78.0 (69.0; 89.0)	83.0 (71.0; 97.5)	0.029
Height, cm *(Me (Q1; Q3))*	170.0 (164.0; 177.5)	167.0 (161.5; 177.5)	0.047
BMI, kg/m² *(Me (Q1; Q3))*	26.3 (23.7; 29.7)	28.7 (25.2; 33.0)	0.002
BMI, kg/m², WHO classification, *N* (%)			
18.5–25.0 normal	626 (36.1)	13 (22.0)	0.026
25.0–30.0 overweight	670 (38.6)	22 (37.3)	0.840
30.0–35.0 obesity I	297 (17.1)	11 (18.6)	0.764
35.0–40.0 obesity II	101 (5.8)	3 (5.1)	0.821
>40.0 morbid obesity	25 (1.4)	10 (16.9)	<0.001
16–18.5 weight deficit	15 (0.9)	0 (0.0)	0.464
Comorbidities, *N* (%)			
Hypertension	306 (17.6)	14 (23.7)	0.229
CHD	122 (7.0)	10 (16.9)	0.004
NYHA class I heart failure	5 (0.3)	1 (1.7)	0.072
NYHA class II heart failure	16 (0.9)	0 (0.0)	0.494
NYHA class III heart failure	1 (0.1)	0 (0.0)	0.808
History of ACVA	15 (0.9)	0 (0.0)	0.494
Diabetes mellitus	62 (3.6)	0 (0.0)	0.138
Score, points, *Me (Q1; Q3)*			
CCI	2 (1; 3)	2 (2; 3)	0.144
CHA_2DS_2-VASc	1 (0; 1)	1 (0; 2)	0.029
HAS-BLED	0 (0; 1)	1 (0; 1)	<0.001
Medications, *N* (%)			
Amiodarone	214 (12.3)	3 (5.1)	0.093
β-blockers (bisoprolol)	1734 (100)	59 (100)	—
Procedures			
RF pulmonary vein isolation, *N* (%)	1503 (86.7)	54 (91.5)	0.283
RFA of the cavo-tricuspid isthmus, *N* (%)	60 (3.5)	1 (1.7)	0.456
RF AV node modification, *N* (%)	129 (7.4)	3 (5.1)	0.505
RFA of arrhythmogenic substrate for atrial extrasystoles, *N* (%)	42 (2.4)	1 (1.7)	0.729
Duration of procedure, minutes *(Me (Q1; Q3))*	115.0 (70.0; 155.0)	130.0 (102.5; 165.0)	0.016
Electrical cardioversion during procedure, *N* (%)	572 (33.0)	21 (35.6)	0.676
History of CA, *N* (%)	25 (1.4)	7 (11.9)	<0.001
Average length of hospital stay, days *(Me (Q1; Q3))*	4 (3; 7)	8 (4.5; 11)	<0.001
PSA characteristics			
Frequency of RASS – 1 to – 2 sedation, *N* (%)	1151 (66.4%)	37 (62.7%)	0.558
Dose of propofol, mg/kg (Me (Q1; Q3))	1.613 (1.282; 2.439)	1.961 (1.389; 2.469)	0.475
Dose of diazepam, mg/kg (Me (Q1; Q3)), *N*=66	*N*=60	*N*=6	0.166
	0.133 (0.121; 0.165)	0.068 (0.058; 0.159)	
Dose of fentanyl, μg/kg *(Me (Q1; Q3))*	1.695 (1.333; 3.333)	2.678 (1.786; 3.659)	0.001
Frequency of NRS >3, *N* (%)	162 (14.1%)	20 (54.1%)	<0.001
Frequency of RASS –2 to –3 sedation, *N* (%)	582 (33.6%)	22 (37.3%)	0.552
Dose of propofol, mg/kg *(Me (Q1; Q3))*	2.469 (2.151; 2.857)	2.381 (2.026; 2.730)	0.376
Dose of diazepam, mg/kg *(Me (Q1; Q3))*, *N*=39	*N*=36	*N*=3	0.091
	0.138 (0.125; 0.162)	0.192(0.168; 0.248)	
Dose of fentanyl, μg/kg *(Me (Q1; Q3))*	2.353 (1.961; 2.837)	2.395(1.971; 2.828)	0.687

ciated with longer hospital stay (1.36; 1.23;1.49) and higher costs (1.16; 1.12;1.19) [30]. D. J. Friedman et al. showed that obesity (AOR: 1.35; 95% CI: 1.09–1.68; *P*=0.005) was associated with an increased risk of cardiac perforation [47]. In our study, BMI >30.0 kg/m² increased the odds of developing PC 1.9-fold compared to patients with BMI <30.0 kg/m² (Table 5).

Pain with a NRS intensity score >3 increased the odds of developing PC 4.3-fold compared to patients with a NRS score <3 (Table 5). Assessment of intraprocedural nociception in patients with

Table 4. Comparison of groups of patients with and without PSA complications.

Parameter	Values in patients		*P*-value
	without PSAC, *N*=1780	with PSAC, *N*=13	
Age, years *(M±SD, Me (Q1; Q3))*	58.7±12.4 61.0 (52.0; 68.0)	61.7±12.1 64.0 (53.0; 69.0)	0.591
Age groups, years (*N* (%))			
Under 60	823 (46.2)	6 (46.2)	1.000
60–64	306 (17.2)	1 (7.7)	0.365
65–69	337 (18.9)	3 (23.1)	0.700
70–74	210 (11.8)	1 (7.7)	0.648
75–79	77 (4.3)	1 (7.7)	0.548
80–84	23 (1.3)	0 (0.0)	0.679
85 and older	4 (0.2)	1 (7.7)	—
Female sex, *N* (%)	898 (50.4)	7 (53.8)	0.807
Weight, kg *(Me (Q1; Q3))*	78.0 (69.0; 90.0)	85.0 (80.0; 100.0)	0.062
Height, cm *(Me (Q1; Q3))*	170.0 (164.0; 177.0)	174.0 (160.0; 182.0)	0.699
BMI, kg/m² *(Me (Q1; Q3))*	26.4 (23.7; 29.8)	25.8 (24.8; 37.2)	0.216
BMI, kg/m², WHO classification, *N* (%)			
18.5–25.0 normal	635 (35.7)	4 (30.8)	0.713
25.0–30.0 overweight	689 (38.7)	3 (23.1)	0.250
30.0–35.0 obesity I	306 (17.2)	2 (15.4)	0.864
35.0–40.0 obesity II	102 (5.7)	2 (15.4)	0.135
>40.0 morbid obesity	33 (1.9)	2 (15.4)	<0.001
16–18.5 weight deficit	15 (0.8)	0 (0.0)	0.746
Comorbidities, *N* (%)			
Hypertension	315 (17.7)	5 (38.5)	0.051
CHD	130 (7.3)	2 (15.4)	0.265
NYHA class I heart failure	6 (0.3)	0 (0.0)	0.843
NYHA class II heart failure	16 (0.9)	0 (0.0)	0.731
NYHA class III heart failure	1 (0.1)	0 (0.0)	0.909
History of ACVA	15 (0.8)	0 (0.0)	0.746
Diabetes mellitus	62 (3.5)	0 (0.0)	0.492
Scores, points *(Me (Q1; Q3))*			
CCI	2 (1; 3)	2 (2; 3)	0.089
CHA_2DS_2-VASc	1 (0; 1)	1 (0; 1)	0.637
HAS-BLED	0 (0; 1)	0 (0; 1)	0.694
Medications, *N* (%)			
Amiodarone	217 (12.2)	0 (0.0)	0.179
β-blockers (bisoprolol)	1780 (100)	13 (100)	—
Procedures			
RF pulmonary vein isolation, *N* (%)	1549 (87.0)	8 (61.5)	0.007
RFA of the cavo-tricuspid isthmus, *N* (%)	59 (3.3)	2 (15.4)	0.016
RF AV node modification, *N* (%)	129 (7.2)	3 (23.1)	0.028
RFA of arrhythmogenic substrate for atrial extrasystoles, *N* (%)	43 (2.4)	0 (0.0)	0.571
Duration of procedure, minutes *(Me (Q1; Q3))*	120.0 (70.0; 155.0)	170.0 (145.0; 260.0)	<0.001
Electrical cardioversion during procedure, *N* (%)	586 (32.9)	7 (53.8)	0.110
History of CA, *N* (%)	30 (1.7)	2 (15.4)	—
Average length of hospital stay, days *(Me (Q1; Q3))*	5 (3; 7)	4 (3; 10)	0.811
PSA characteristics			
Frequency of RASS –1 to –2 sedation, *N* (%)	1183 (66.5)	5 (38.5)	0.033
Dose of propofol, mg/kg *(Me (Q1; Q3))*	1.613 (1.290; 2.439)	1.695 (1.429; 2.000)	0.821
Dose of diazepam, mg/kg *(Me (Q1; Q3))*, *N*=66	*N*=65 0.130 (0.120; 0.164)	*N*=1 0.286	—
Dose of fentanyl, μg/kg *(Me (Q1; Q3))*	1.724 (1.351; 3.333)	7.000 (4.237; 7.500)	0.001
*Frequency of NRS >3, *N* (%)	178 (15.0)	4 (80.0)	<0.001
Frequency of RASS –2 to –3 sedation, *N* (%)	597 (33.5)	8 (61.5)	0.033
Dose of propofol, mg/kg *(Me (Q1; Q3))*	2.469 (2.151; 2.857)	2.300 (2.000; 2.417)	0.183
Dose of diazepam, mg/kg *(Me (Q1; Q3))*, *N*=39	*N*=38 0.143 (0.125; 0.163)	*N*=1 0.303	—
Dose of fentanyl, μg/kg *(Me (Q1; Q3))*	2.353 (1.961; 2.817)	3.201 (2.663; 3.551)	0.021

Note. * — for patients with RASS sedation level from –1 to –2.

arrhythmias is challenging. The main limitation for nociception monitoring with registration of autonomic variability parameters in such patients is cardiac arrhythmia, and nociception monitoring based on registration of electrophysiological parameters (EEG, EMG) demonstrates the possibility of assessing the response to a nociceptive stimulus only under general anesthesia. Near-infrared functional spectroscopy has shown promising results for the objective measurement of intraoperative nociception in CA patients under general anesthesia, with cortical meas-

Table 5. Risk factors for the procedural complications of CA in patients with atrial arrhythmias.

Predictors	Frequency in patients, *N* (%)		Crude OR (95% CI)		Adjusted OR (95% CI)	
	with PC, *N*=59	without PC, *N*=1734	Value	*P*-value	Value	*P*-value
Age > 69 years	23 (39.0)	294 (17.0)	3.129 (1.827; 5.359)	<0.001	3.081 (1.764–5.383)	<0.001
BMI > 30 kg/m²	24 (40.7)	423 (24.4)	2.125 (1.250; 3.614)	0.005	1.919 (1.094; 3.363)	0.023
CHA_2DS_2-VASc > 1 points	19 (32.2)	335 (19.3)	1.984 (1.134; 3.469)	0.016	—	—
Duration of procedure > 117 minutes	38 (64.4)	864 (49.8)	1.822 (1.061; 3.130)	0.030	—	—
NRS > 3 points	20 (33.9)	203 (11.7)	4.976 (2.835; 8.736)	<0.001	4.317 (2.390; 7.800)	<0.001
Fentanyl dose > 2.37 μg/kg	36 (61.0)	692 (39.9)	2.357 (1.385; 4.012)	0.002	—	—
History of CA	7 (11.9)	25 (1.4)	9.202 (3.808; 22.238)	<0.001	10.276 (4.006–26.354)	<0.001
History of CHD	10 (16.9)	122 (7.03)	2.697 (1.333; 5.455)	0.006	—	—

Table 6. Risk factors for PSA complications in patients with CA for atrial arrhythmias.

Predictors	Frequency in patients, *N* (%)		Crude odds ratio (COR)		Adjusted odds ratio (AOR)	
	with PC, *N*=13	without PC, *N*=1780	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
BMI > 35 kg/m²	4 (30.8)	135 (7.6)	5.416 (1.646–17.816)	0.005	4.955 (1.485–16.535)	0.009
Duration of procedure > 142 min	11 (84.6)	576 (32.4)	11.497 (2.540–52.037)	0.002	11.070 (2.440–50.228)	0.002

urements potentially more accurate than current assessment methods [48].

A. Vevecka et al. demonstrated in a multivariate analysis that obstructive sleep apnea was the only independent predictor of NILV [49]. S. D'Souza et al. found that obesity was associated with a 2.6-fold increased risk of respiratory complications (AOR: 1.39; 95% CI: 1.20–1.62) [30]. In a study by L. Foerschner et al., BMI > 30.1 kg/m² was a predictor of the need for NILV/ILV (AOR: 1.6, *P*=0.03) [40]. In our study, BMI > 35 kg/m² increased the odds of PSA complications by 4.95-fold, while procedure duration >142 min increased the odds by 11.0-fold compared to lower values of these parameters (Table 6).

Conclusion

Independent predictors of CA complications were patient-related factors such as BMI > 30.0 kg/m², age > 69 years and CA procedure-related factors such as duration of CA > 142 min, previous history of CA, and presence of pain with intensity > 3 points on the NRS.

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Full Outline of UnResponsiveness (FOUR) Scale: a Multicenter Validation Study of the Psychometric Properties of the Approved Russian Version

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Summary

Assessment of the individual level of consciousness on admission of a patient with brain injury to the intensive care unit (ICU) is a priority task and a mandatory step in the overall assessment of neurological status. The Full Outline of UnResponsiveness (FOUR) scale, developed at the Mayo Clinic (USA) in 2005, is a widely used tool for comprehensive assessment of patients with altered state of consciousness. The lack of a validated Russian-language version of the FOUR scale has hindered its widespread use in clinical practice. Therefore, the official Russian version of the FOUR scale was developed and adapted for use in Russia after the first stage of the validation study (linguistic and cultural adaptation).

Aim. To evaluate the psychometric properties of the Russian version of the FOUR scale for comprehensive assessment of patients in altered state of consciousness.

Materials and Methods. As part of a prospective multicenter validation study, the psychometric properties of the scale (reliability, validity, and sensitivity) were evaluated in a group of 171 adult patients with altered conscious state of various etiologies, such as ischemic and hemorrhagic stroke, neuroinflammatory conditions, and traumatic brain injury. Patients' responses were assessed on the first day of ICU stay and 2–3 days later by two ICU neurologists with at least three years of experience.

Results. High levels of validity and reliability were obtained for the Russian version of the FOUR scale for comprehensive assessment of unresponsive patients, including Spearman's rank correlation coefficient R=0.99 (P<0.0001), Cohen's κ=0.77 (P<0.001), Cronbach's α=0.87 (P<0.0001). Regarding the sensitivity of the FOUR scale, no significant changes were found after comprehensive assessment of unresponsive patients on day 1 in the ICU and 2–3 days later (Wilcoxon test, p=0.906). There was a good correlation between the FOUR and Glasgow Coma Scale scores used to assess patients with altered state of consciousness, confirming the validity of the test with R=0.91 (P<0.0001).

Conclusion. The Russian version of the FOUR scale for comprehensive assessment of unresponsive patients is a valid, reliable, and sensitive clinical tool. Sufficiently verified level of psychometric properties allows its authorized use in Russia and other Russian-speaking countries. The scale is available for download via QR code and at the website of the International Scales and Questionnaires Validation Group at the Research Center for Neurology.

Keywords: FOUR scale; Full Outline of UnResponsiveness; coma; altered state of consciousness; validation; resuscitation

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

Introduction

Clinical assessment of coma patients is an important diagnostic skill for medical professionals. The scales used to assess neurological patients in critical illness have been developed to standardize the examination, objectively evaluate the results and, obviously, facilitate communication between specialists. The most commonly used scale is the Glasgow Coma Scale (GCS) [1]. The GCS is a classic scale developed in 1974 to assess the severity of impaired consciousness in patients with brain injury admitted to intensive care. It is an algorithm consisting of a series of tests, including eye opening, verbal response, and motor response [2]. Although the authors of the GCS reported evidence of the practical reliability of the scale, later difficulties in its application emerged, and the arsenal of equipment available to maintain vital functions in the ICU necessitated the expansion of the diagnostic items to address the severity of impaired wakefulness [3–4].

Thus the verbal component of the GCS assessment cannot be tested during tracheal intubation. Some clinicians use the lowest possible score, while others extrapolate other neurological findings to the verbal response [5]. Second, changes in respiratory pattern and the need for ventilatory support may reflect the depth of coma, but GCS does not include these clinical parameters [6]. Third, the GCS may not reflect minor changes on neurological examination [7].

Due to the need for a new tool, an improved scale to assess the status of a coma patient, the Full Outline of UnResponsiveness (FOUR) detailed assessment scale for unresponsive patients was developed [8].

The score consists of 4 components to be tested: eye response, motor response, brainstem reflexes, and respiration [9]. The introduction of this scale into clinical practice has shown a high degree of consistency in the interpretation of scores by practitioners of different specialties, including emergency department nurses [10].

This scale has been linguistically and culturally adapted and validated in many countries [11–12]. The lack of a validated version of the scale complicates its use in Russia. For successful standardized clinical and relevant use of the scale, the adapted Russian-language version must undergo all necessary validation stages.

After the development of the official Russian version of the Full Outline of UnResponsiveness scale [13–15], the second and final stage of the validation study was conducted.

The aim of this study was to evaluate the psychometric properties of the Russian version of the FOUR scale.

Materials and Methods

Patients were prospectively recruited at the Research Center for Neurology (Moscow), S. P. Botkin City Clinical Hospital (Moscow), N. V. Sklifosovsky Research Institute of Emergency Medicine (Moscow), V. A. Almazov National Medical Research Center of the Ministry of Health of Russia (St. Petersburg), and Clinical Institute of Brain (Yekaterinburg) in the period from June 2018 to July 2021.

According to the inclusion and exclusion criteria (Table 1), 176 neurological patients over 18 years of age with different levels of impaired consciousness (coma, stupor, obtundation), as well as patients in full consciousness participated in the study.

During the inter-evaluation period, 5 patients were excluded from the study: three due to death, one due to sedation specifics, and one patient was transferred to a multidisciplinary hospital due to bleeding.

The final group consisted of 171 patients (87 males and 84 females). Severity of altered consciousness was clinically assessed on the first day of hospitalization (concurrent with the first GCS assessment) by two ICU neurologists with at least 3 years of experience.

Validation procedure. The second stage of validation of international scales involves the study of psychometric parameters such as reliability, validity, and sensitivity. These parameters of the FOUR scale were evaluated with the participation of two experienced neurologists. The scores of the questionnaire at the first, second and third examination by the first physician were designated as «A1», «A2» and «A3», and at the examination by the second physician as «B1», respectively.

Psychometric parameters. Based on the principles of validation of tests, questionnaires, and

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Age ≥ 18 years. • Hospitalization in the intensive care unit with the following types of altered consciousness: stupor, obtundation, coma, as well as those in full consciousness. • Signed informed consent from patient or patient's representative. • Documented neurological conditions such as ischemic/hemorrhagic acute cerebrovascular accident (including subarachnoid hemorrhage), traumatic brain injury, infectious diseases of the central nervous system (meningitis, encephalitis, etc.), acute neuromuscular diseases (Guillain-Barré syndrome, myasthenic crisis, etc.), and others.	The effect of sedatives or neuromuscular blockers at the time of assessment using the specified scales. In this case, it was necessary to wait one maximum half-life (during the baseline and repeated assessment over the next 3 days after establishing the fact of taking these medications).

scales, the following psychometric parameters were evaluated: test-retest and inter-rater reliability, internal consistency, criteria-related and content validity, and sensitivity [16].

The content validity study was conducted by interviewing five experts (neurologists with at least 8 years of experience) to determine how well the content of the scale matches the tasks for which it is used. The assessment was made on a 10-point scale.

The study of the sensitivity of the scale included a comparison of the results of the initial and final examinations of the patients (A1–A3). The hypothesis about the effectiveness of the scale in detecting changes in clinical parameters was tested.

Statistical analysis of data. An adequate sample size was calculated according to generally accepted recommendations [17]. The sample size, which amounted to 171 people, provided the necessary level of its representativeness.

The following methods of statistical data analysis were used to study the psychometric parameters of the scale: retest reliability and criterion-related validity (with GCS scores) were assessed using the Spearman correlation test, inter-rater reliability was assessed using Cohen's kappa, internal consistency was assessed using Cronbach's alpha coefficient and intraclass correlation coefficient, and sensitivity was assessed using the Wilcoxon test. The attainment of a threshold level of inter-rater consistency, Cohen's kappa, was used as the endpoint. The size of the differences was chosen at the level of 0.4 points on the scale under study. The power level was 0.8. In all cases of statistical hypothesis testing, $P \leqslant 0.05$ was considered significant. Statistical analysis of data was performed using SPSS Statistics 22 software (IBM Corp., Chicago, USA).

Results

Patient characteristics. Based on the neurological profile, the studied patients (N=171, mean age 63.0±16.8 years) were divided into the group with brain injury (N=164) and the group with peripheral nervous system injury (N=7). The etiology of brain injury is shown in Figure.

Other causes included inflammatory diseases of the brain and meninges (encephalitis and meningitis) (3/164), closed traumatic brain injury (3/164), demyelinating diseases (3/164), cerebrovascular disease (2/164), epilepsy (2/164), brain tumors (2/164), consequences of cardiac arrest (1/164), consequences of aorto-coronary bypass surgery (1/164), opportunistic infection with human immunodeficiency virus (1/164), toxic encephalopathy with heroin addiction (1/164).

The peripheral nerve injury group consisted of patients with Guillain–Barré syndrome (6/7) and myasthenic crisis (1/7).

The median and interquartile range *(Me [IQR])* of the FOUR score at the first visit was 16.0 [11.25–16.0] points. Full consciousness was noted in 94 patients (55.0%), mild obtundation in 6 patients (3.5%), severe obtundation in 14 patients (8.2%), stupor in 26 patients (15.2%), and coma in 31 patients (18.1%).

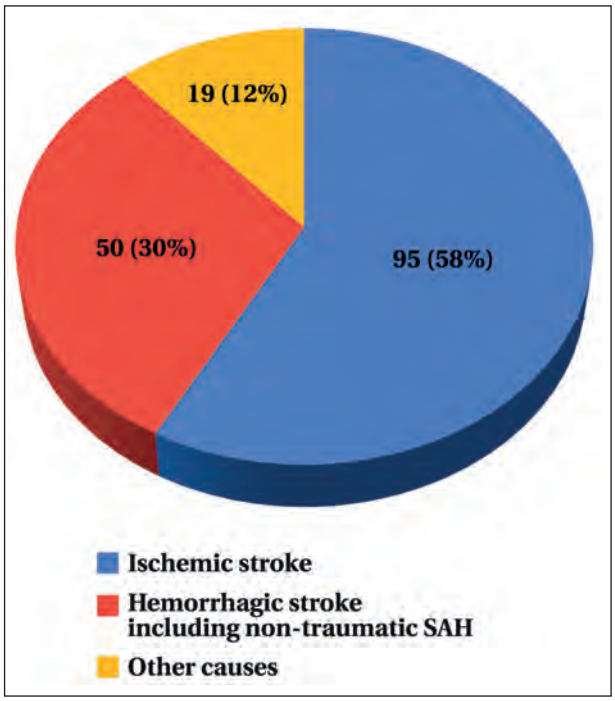

Fig. Distribution of patients in the CNS injury group (N=164) by ethology.

Psychometric properties of the Russian version of the FOUR scale.

Reliability. The Spearman correlation coefficient between the results of repeated examinations in the study of retest reliability was R=0.99 (P<0.0001), which corresponds to a high level of stability of the scale to time-related errors.

Cohen's kappa coefficient was κ=0.77 (P<0.0001), which supports significant inter-rater agreement in independent assessment of the FOUR scale. When examining the discrepancy between the scores of each of the E, M, B, and R score components, significant and balanced scores were obtained (Table 2).

The internal consistency study of the FOUR scale showed that the Cronbach's alpha coefficient was α=0.87 (P<0.0001) with an intraclass correlation coefficient (ICC) of 0.87 (P<0.0001), indicating a strong balance of scale items.

When examining the criterion validity between the FOUR and GCS scores, a significant correlation of R=0.912 (P<0.0001) was found.

Based on expert evaluation, the content validity was high, scoring 8.8 out of 10.

Sensitivity of the Russian version of the FOUR scale. Comparing the score of the FOUR scale at the first examination (16.0 [11.2–16.0] points) and

Table 2. Inter-rater agreement on the components of the Full Outline of UnResponsiveness (FOUR) score.

Inter-rater agreement	The components of Full Outline of UnResponsiveness (FOUR) score				
	Eye response (E)	Motor response (M)	Brainstem reflexes (B)	Respiration (R)	Total
Cohen's kappa (threshold value ⩾0.7)	0.847	0.875	0.807	0.92	0.770
P-value			<0.0001		

Table 3. Psychometric parameters of the Russian version of the Full Outline of UnResponsiveness (FOUR) scale.

Parameter	Elements	Assessment method	Threshold value	*Result*	
				Value	P-value
Reliability	Internal consistency (A1)	Cronbach's alpha	0.8 and more	0.87	<0.0001
		Intraclass correlation coefficient	0.8 and more	0.87	<0.0001
	Inter-expert agreement	Cohen's kappa	0.7 and more	0.77	<0.0001
	Test-retest reliability (A1–A2)	Spearman correlation coefficient	0.7 and more	0.73	<0.0001
Validity	Criterion-related validity	Spearman correlation coefficient	0.7 and more	0.91	<0.0001
	Content validity	Expert evaluation	7/10 and more	8.8/10	—
Sensitivity	Sensitivity (A1–A3)	Wilcoxon test	*P*<0.05	0.118	0.906

at the final examination (16.0 [11.0–16.0] points), no significant change in the scores was found (Wilcoxon criterion, *P*=0.906), which may indicate that the patients' condition remained stable during this period between assessments (2–3 days).

Discussion

The FOUR scale is an effective tool for rapid and standardized assessment of acute impairment of consciousness. It can be used to assess the degree of altered state of consciousness [18]. The modalities presented in this scale can be used for assessment not only by subspecialists such as neurologists and intensivists, but also by trainees and nurses [19]. The Full Outline of Unresponsiveness scale is easy to use and remember, quick to implement, reliable in different settings, and provides physicians with sufficient information about the patient's condition to determine management strategies [20]. Unfortunately, the lack of a Russian version limited the use of the scale, necessitating its development and validation.

Previously, at the first stage of validation, we conducted forward and backward translations of the scale, then approved the final text of the scale, taking into account all cultural and linguistic peculiarities of Russian medical terminology. The official Russian version of the FOUR scale was published in 2019 in the journal Annals of Clinical and Experimental Neurology [13].

However, the first step is not sufficient for the reliable use of the developed version of the scale in clinical and research practice. Only the evaluation of psychometric parameters with the use of statistical methods of analysis will allow to ensure that the use of this assessment tool will provide objective clinical data and the same result as the use of the original version. In addition, the comparison of the obtained results with those of international researchers, as well as the global acceptance of the results obtained in Russia and in the Russian population, will be possible only after the examination of psychometric parameters.

In the second step of validation we conducted a multicenter study, which included 171 patients with various levels of altered consciousness (coma, obtundation, stupor), as well as patients in full consciousness. After data collection, significant psychometric parameters of the Russian version of the scale were obtained (Table 3).

The examination using the FOUR scale was performed by two experienced neurologists, which made it possible to assess the inter-expert reliability of the scale in a heterogeneous population.

The inter-rater reliability for the total score of the FOUR scale was significant (κ=0.77, *P*<0.0001), which is a positive result compared to previous studies [21]. This could be because the current study involved two experienced neurologists working with patients in the neurological intensive care unit, which significantly improved the examination's accuracy.

This also confirms the importance of practical training of physicians in the use of the scale to reduce bias.

The scale showed a high resistance to time-related errors (Spearman correlation coefficient *R*=0.99, *P*<0.0001), which indicates the short time required for assessment and is one of the advantages of the scale, along with its accessibility and simplicity. Thus, the tool can be used not only in scientific research but also in routine clinical practice.

The elements of the scale were found to be highly balanced with α=0.87 (*P*<0.0001) and ICC=0.87 (*P*<0.0001), which also emphasizes the reliability of the scale.

The scores of the Russian version of the FOUR scale were highly consistent. All of this confirms the primary goal of the developed FOUR scale, which is to meet the need for simple and rapid assessment of all major neurological signs in patients with acute disorders of consciousness. The scale does not assess the verbal modality, but it provides a good assessment of eye movements, brainstem reflexes and respiratory pattern in ventilated patients.

The Russian version of the FOUR scale is available in the appendix and on the website of the International Scale and Questionnaire Validation Group of the Research Center for Neurology https://neurology.ru/o-centre/struktura/institut-neyroreabilitatsii-i-vosstanovitelnykh-tekhnologiy/gruppa-validatsii-mezhdunarodnykh-shkal-i-oprosnikov/?ysclid=lo46dsgpr9826437705.

Conclusion

We completed all necessary validation steps for the Russian version of the FOUR (Full Outline of Unresponsiveness) scale. The scale psychometric properties of the scale were evaluated and found to be highly reliable and valid. This version is recommended for use in research and clinical settings.

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RICD: Russian Intensive Care Dataset

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Summary

In the era of healthcare digital transformation, the scientific community faces the need for structured and available datasets for research and technological projects in the field of artificial intelligence, related to the development of new diagnostic and treatment methods.

Objective: to develop a dataset containing anonymized medical data of all patients treated at the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology (FRCCR), and provide access for doctors and scientists of FRCCR and other centers to structured patient data for subsequent analysis and research.

Materials and Methods. The FRCCR medical information system and the tools «Asclepius», PL/SQL, Microsoft Office Excel, Power Query M, Microsoft PowerBI, Open data editor, and Python were used for data collection and representation. To provide open access to the dataset and protect the personal data of patients, the information was anonymized.

Results. We introduce the RICD (Russian Intensive Care Dataset, https://fnkcrr-database.ru/) — the first dataset of intensive care patients in the Russian Federation, developed at FRCCR based on advanced principles and methods used in international open database projects — «eICU Program» from Philips Healthcare, «MIMIC-IV», and «MIMIC-III». The developed dataset contains information on 7,730 hospitalizations of 5,115 patients (including readmissions), covering data from 3,291 hospitalizations in the intensive care units (ICUs). The total number of records in the RICD exceeds 14 million. The RICD presents medical-anthropometric data, patient movement within the institution, diagnoses, information on therapy provided, results of laboratory tests, scale assessments, and outcomes of hospitalization. RICD also contains data on several vital parameters collected from bedside monitors and other equipment of ICUs, with up to 10 evaluations per hour.

Conclusion. The RICD allows for in-depth analysis and research of clinical practices in intensive care, enabling the development of clinical decision support tools and the application of machine learning methods to enhance diagnostic tools and improve patient outcomes. With its accessibility and detailed data structure, the dataset serves as a valuable tool for both scientific research and practical applications in intensive care.

Keywords: dataset; critically ill patients; intensive care; artificial intelligence; machine learning; clinical decision support systems; https://fnkcrr-database.ru/

Conflict of interest. The authors declare a potential conflict of interest in case of commercialization of the dataset presented in this article.

Introduction

In contemporary medicine, there is a rapid development of electronic healthcare systems, which allow data collected during routine clinical practice to be organized and stored in medical institutions globally. These systems are particularly emphasized in the field of critical care due to the necessity for continuous monitoring of patients' vital functions in intensive care units (ICUs). This approach results in the generation of substantial data, which, upon detailed examination, facilitate the improvement of clinical practice. Projects such as «MIMIC-III» [1] (a database representing data from over 40,000 patients admitted to the ICU of the Beth Israel Deaconess Medical Center, USA, between 2001 and 2012) and «MIMIC-IV» [2] (data on hospitalizations of 69,653 patients from the same center, covering 2008–2019), as well as «eICU» [3] (a multicentric database comprising de-identified medical data from over 200,000 ICU admissions across numerous US medical centers between 2014 and 2015), illustrate how valuable information collected in ICUs can be utilized for scientific research and the development of new diagnostic and treatment methods. As of 2024, over 1,500 scientific publications have utilized data from the «MIMIC-III», «MIMIC-IV», and «eICU» projects, including journals such as Critical Care [4], Nature [5], Lancet [6], JAMA [7], BMC Anesthesiology [8].

Other international projects contributing significantly to clinical research include «HiRID» [9] (36,098 ICU hospitalizations in Switzerland), «AmsterdamUMCdb» [10] (23,106 ICU hospitalizations

in the Netherlands), and the database «The Children's Hospital at Zhejiang University School of Medicine» [11] (13,941 pediatric ICU admissions in China). These repositories provide unique data on patient care and clinical outcomes. However, it should be noted that due to significant differences in healthcare systems across countries, the use of foreign open database projects in domestic practice is limited. In 2023, the Siberian State Medical University introduced Russia's first clinical data repository, «SibMED Data Clinical Repository», encompassing anonymized data from more than 20,000 hospitalizations in 10 multi-specialty clinics of SibSMU [12]. As of 2024, this repository does not include monitored data of ICU patients, which limits the depth of research in intensive care.

Currently, the integration of artificial intelligence (AI) technologies into medical practice opens new possibilities for diagnosis, treatment, and monitoring of patient conditions, especially relevant for patients in intensive care settings. AI technologies encompass a broad range of methods and approaches, including neural networks, machine learning techniques, and expert systems. These tools are uniquely capable of analyzing complex and extensive datasets, including medical images, textual documents (such as diary entries, discharge summaries, clinical notes, and reports), and databases of medical parameters [13]. One of the most promising applications of AI in critical care is the real-time monitoring and analysis of patient conditions. AI technologies can predict the development of complications and adverse outcomes, identifying life-threatening conditions requiring medical staff attention, thereby becoming crucial elements of clinical decision support systems, helping to reduce the number of medical errors and improve the quality of medical care provided [14, 15]. The development of such systems requires the creation of large, structured, and accessible datasets.

The Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology (FRCCR) has implemented a concept of «digital» ICU, which entails the collection and storage on organizational servers of all data from ICU patients, including continuously monitored parameters, making it accessible for analysis. The project's goal is to develop a dataset containing anonymized medical data of all patients treated in the FRCCR, providing physicians and researchers from the FRCCR and other centers access to structured patient data for further analysis and research.

Materials and Methods

Data acquisition. The data were obtained using standard tools of the medical information system (MIS) «Asclepius», based on an Oracle 11g relational database management system. The primary dataset was extracted from various MIS modules (registration, clinical, laboratory, pharmacy, etc.) using the «Query Builder» virtual module. Additional queries were performed in the PL/SQL language directly from the relational database management system. The data extraction period was from December 2017 to July 2023. Laboratory investigation results were partly acquired by generating reports from the «Alisa» laboratory information system. Data structuring was facilitated by creating macros in the «VBA» language in Microsoft Office Excel 2021 and utilizing the «Power Query M» programming language. For demonstrating the analytical capabilities of the dataset, the BI system «Microsoft PowerBI,» the programming language «Python» in the «PyCharm 2023.2.4» environment, and IBM SPSS Statistics 27.0 were used. For standardization, directories were created to unify and standardize data from various sources. The final dataset files were created using the «Frictionless data» technology, implemented in the «Open data editor» program.

Anonymization and pseudonymization. All data were depersonalized (anonymized) to facilitate open access. The data anonymization methodology was developed based on the National Standard of the Russian Federation «Health informatics. Pseudonymization» GOST R 55036-2012/ISO/TS 25237:2008 (approved by the order of the Federal Agency on Technical Regulating and Metrology dated October 29, 2012, No. 585-st) and international open database projects. The anonymization approach was approved by the local Ethics Committee (No. 4/23/2 dated 20.12.2023). Direct and indirect patient identifiers were removed. To maintain the informativeness of the dataset, new parameters were created in each table, reflecting the timing of the investigation/examination or parameter assessment (post_admission_days and post_admission_hours). Additionally, a pseudonymization procedure was

Table 1. Descriptive characteristics of hospital and ICU patients in the RICD.

Parameter	Hospital	ICU
Number of hospitalizations	4339	3291
Number of patients*	3033	2562
Age, mean *(SD)*	59.2 (15.2)	57.8 (17.7)
Sex, male (%)	2385 (55.0)	1864 (56.6)
Length of stay, days *(Q1–Q3)*	14.0 (14.0–18.0)	32.0 (22.0–50.0)

Note. * — the number of patients exceeds 5115 because some of the patients were hospitalized in different periods of time in both the intensive care unit and the hospital.

Table 2. Brief description of the RICD tables.

Table	Number of entries	Description
1. all_patients	7730	Medical and anthropometric characteristics of patients, patient movement within the institution, hospitalization outcomes
2. ICD10_diagnoses	1058929	Diagnoses of patients according to ICD-10 classification
3. therapy_prescriptions	550880	Therapeutic assignments
4. clinical_notes	304680	Routine patient assessment and ICD-10 diagnoses
5. monitoring_data	12198188	Monitored parameters (vital signs and fluid balance parameters)
6. all_scales	19297	Scale scores
7. detailed_sofa	14859	Detailed structure of the SOFA scale score
8. complete_blood_count	43584	Complete blood count test results
9. urinalysis	37307	Results of urinalysis
10. blood_biochemistry	65882	Results of blood biochemical analysis
11. urine_biochemistry	1389	Results of biochemical analysis of urine
12. coagulation_profile	33727	Results of hemostasis system evaluation
13. acid_base_balance	6185	Results of evaluation of acid-base balance and blood gases
14. antibiotic_resistance	6794	Results of assessment of antibiotic resistance
15. bacteria_culture_test	6101	Results of bacteria culture tests
16. cerebrospinal_fluid_analysis	1021	Cerebrospinal fluid analysis

conducted, replacing identifying data (patient ID, hospitalization ID/medical history number) with pseudonyms (unique identifiers: new_patient_id, new_hosp_id), which cannot be linked to the original data without additional information stored separately.

Dataset Description

Descriptive characteristics. The RICD contains data on 7,730 hospitalizations of 5,115 patients admitted to the FRCCR during the 2017–2023 period, including 3,291 hospitalizations in the ICU (Table 1). The average age of ICU patients was 57.8 years (SD 17.7, range from 19 to 97 years), and the median duration of ICU stay was 32 days (22–50), with a maximum of 320 days. During the entire period, 405 deaths were reported. The total number of entries in all RICD tables is 14,356,553.

Data format. The dataset has been presented in several formats:

1. .csv files, interconnected through key fields (frictionless data);
2. .db format (for SQLite);
3. .pbix format (for Microsoft PowerBI system).

Additionally, metadata were provided in .json format. RICD files can be uploaded into any relational database or BI-system.

Dataset structure. The dataset comprises 16 interconnected tables (detailed description is provided in the Supplement). The unifying identifiers of all tables are: new_patient_id (modified patient ID) and new_hosp_id (modified medical history ID).

The tables contain patients' medical-anthropometric data, information about their movement within the institution, diagnoses, data on the therapy provided, results of laboratory tests, scale assessments, vital and fluid parameters assessed dynamically, and hospitalization outcomes (Table 2).

In the RICD, 85% of all entries represent assessments of monitored vital signs of ICU patients (results from pulse oximetry (SpO_2), body temperature, respiratory rate, heart rate, systolic and diastolic BP, average BP, central venous pressure) and fluid balance parameters.

Key features of RICD. 1. Availability of monitored data of ICU patients. RICD is the first dataset in the Russian Federation that presents data collected from bedside monitors and other equipment of the ICU. The sampling frequency (data assessments) is up to 10 assessments per hour.

2. The primary cohort of patients consists of individuals who have been staying in ICUs for extended periods. More than 60% of the patients had a reduced level of consciousness (Glasgow Coma Scale score <15 points).

3. Availability of bacteria culture tests with evaluation of antibiotic resistance.

Technical validation. For the assessment of the developed dataset, an interdisciplinary team was involved, which conducted data validation, evaluated the integrity of data through cross-checking tables, identified acceptable ranges of data values, and determined the completeness of data anonymization.

Access platform. To provide access to the RICD project, a platform (website: https://fnkcrr-database.ru) was developed. The website presents complete information about the RICD project, describes the structure of the dataset, and offers the possibility to obtain a demo version of the dataset, representing the hospitalization data of 10 patients (over 60,000 monitored parameter assessments in the ICU). Additionally, materials on working with RICD biomedical data are available, which can be used to study the dataset and familiarize with possible tools for its analysis.

Applying for access offers the option «participate in the RICD project», which creates a basis for the integration and unification of dataset of various medical centers.

Demonstration of analytical capabilities. Figure 1 presents the parameters of a patient monitored over 15 days of hospitalization at our center. During

the specified period, there are over 1000 assessments for each of the vital parameters (including SpO_2, respiratory rate, and heart rate), dynamics of changes in laboratory parameters, and evaluations based on clinical scales. Moreover, the RICD provides the ability to evaluate the mechanical ventilation status and therapy prescriptions, including the use of vasopressor and inotropic drugs.

Conclusion

The presented RICD dataset enables in-depth analysis and research of clinical practices in intensive care, development of clinical decision support tools and application of machine learning methods to solve diagnostic problems and improve patient outcomes. Due to the accessibility and detailed structuring of the data, the dataset will be a useful tool for both scientific and practical applications. The RICD project is recommended for use by researchers, data science and machine learning specialists, and developers of advanced digital health solutions.

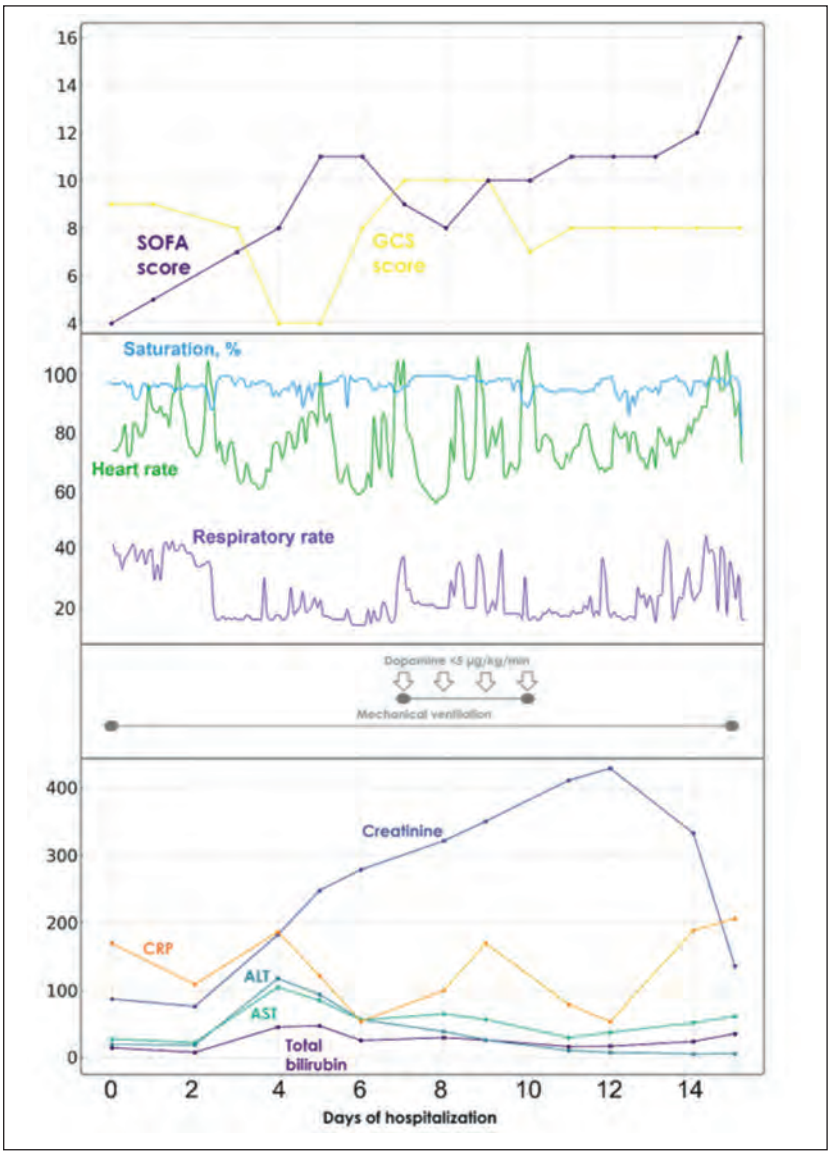

Fig. Parameters monitored during 15 days of the patient's hospitalization at Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology.
Note. Data from tables all_scales, monitoring_data, blood_biochemistry, and detailed_SOFA scores were used.

Supplement. Detailed description of the RICD tables

1. All_patients (7,730 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
sex	Patient gender	—
body	Weight at admission	kg
height	Patient's height at admission	cm
BMI	Body mass index at admission	kg/m²
age	Patient's age at admission	years
patient_condition	Patient's condition at admission	—
transfer	Fact of transfer from another institution	—
adm_year	Year of admission	—
admission_department	Admission department	—
discharge department	Discharge department	—
ICU_patients	ICU patient	—
length_of_stay	Length of stay	days
fatal_outcome	Fatal outcome	—

2. ICD10_diagnoses (1,058,929 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
document_type	Document type	—
diagnosis_type	Diagnosis type	—
ICD_10	ICD-10 code	—

3. therapy_prescriptions (550,880 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
prescription_rus	Prescription in Russian	—
prescription_eng	Prescription in English	—
Time (12:00_am — 11:00_pm)	Estimated time of performance/assignment status	V assigned + completed X canceled – failed

4. clinical_notes (304,680 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
patient_condition	Patient's condition at the time of assessment	—
ICD_10	ICD-10 codes	—

5. monitoring_data (12,198,188 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
parameter	Monitored parameter*	—
unit	Units	—
hour	Evaluation time	—
value	Value	—

***Vital parameters:** saturation (SpO_2), temperature, respiratory rate, heart rate, diastolic BP, systolic BP, mean AP, body mass, glucose, respiratory volume, central venous pressure, BIS, $EtCO_2$. ***Fluid parameters:** fluid intake per os, diuresis, enteral feeding, infusion, liquor, stool/stoma, other intake, other output, gastrostoma, drainages volume, nasogastric tube/vomitus, cystostomy.

6. all_scales (19,297 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
scales	Scales* and evaluation results	—

***Scales:** CRS-R, APACHE II, CHA_2DS_2-VASc, DRS, FIM, FOUR, GRACE, HAS-BLED, NIHSS, POSSUM, SAPS II, SOFA, Barthel, Waterlow (pressure ulcer risk), Geneva score, Glasgow coma scale, Classification of surgical and anesthesia risk (MNOAR), Ashworth scale, Caprini (DVT/PE risk), Palliative performance scale, Rehabilitation routing scale, Rivermead mobility index, Modified Rankin scale, Wells' Criteria for DVT.

7. detailed_sofa (14,859 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
report	Text Conclusion	—
sofa_score	SOFA score	—
FiO_2 %	FiO_2 %	%
PaO_2	PaO_2	mm Hg
PaO_2/FiO_2	PaO_2/FiO_2	—
mechanical_ventilation	Mechanical ventilation	—
platelets	Platelets	10^9/L
GSC_score	Glasgow Coma Scale Score	—
bilirubin	Bilirubin	umol/L
systolic_AP	Systolic blood pressure	mm. hg
diastolic_AP	Diastolic blood pressure	mm. hg
mean_AP	Mean blood pressure	mm. hg
vasoactive_drugs	Use of vasoactive drugs	—
creatinine	Creatinine	umol/L
daily_diuresis	Daily urine output	ml

8. complete_blood_count (43,584 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
WBC	White Blood Cells	10^9/L
RBC	Red Blood Cells	10^{12}/L
HGB	Hemoglobin	g/L
HCT	Hematocrit	%
MCV	Average red blood cell volume	fl
MCH	Average red blood cell content	pg
MCHC	Mean red blood cell hemoglobin concentration	g/l
RDW	Red blood cell distribution width by volume	%
RDW-SD	Standard deviation of red blood cell distribution width by volume	fl
PLT	Platelets	10^9/L
PCT	Thrombocrit	%
MPV	Average platelet volume	fl
PDW	Platelet distribution width by volume	%
NEU%	Neutrophil percentage	%
NEU	Absolute neutrophil count	10^9/L
LYM %	Percentage of lymphocytes	%
LYM	Absolute number of lymphocytes	10^9 /L
MONO %	Percentage of monocytes	%
MONO	Absolute number of monocytes	10^9/L
EOS %	Percentage of eosinophils	%
EOS	Absolute number of eosinophils	10^9/L
BASO %	Percentage Basophil percentage	%
BASO	Absolute basophil count	10^9/L
NRBC%	Percentage of normoblasts	%
NRBC #	Absolute number of normoblasts	10^9/L
%RETIC	Percentage of reticulocytes	%
RETIC	Absolute number of reticulocytes	10^9/L
IRF	Immature reticulocyte fraction	%
MRV	Average reticulocyte volume	fl
band_neutrophil	Band neutrophils	%
segmented_neutrophil	Segmented neutrophils	%
ESR	Erythrocyte sedimentation rate	mm/h

9. urinalysis (37,307 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
urine_color	Urine color	—
clarity/turbidity	Urine transparency	—
specific_gravity	Specific gravity	units
PH	pH of urine	—
protein	Protein	mmol/L
glucose	Glucose	mmol/L
nitrites	Nitrites	—
ketones	Ketones	mmol/L
bilirubin	Bilirubin	umol/L
ascorbic_acid	Ascorbic acid	mmol/L
urobilinogen	Urobilinogen	umol/L
squamous_epithelial_cells	Squamous epithelium	—
transitional_epithelial_cells	Transitional epithelium	—
WBCs	WBCs	—
RBCs	RBCs	—
bacteria	Bacteria	—
hyaline_casts	Hyaline casts	—
nonclassified_casts	Nonclassified casts	—
mucus	Mucus	—

10. blood_biochemistry (65,882 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique Hospitalization ID	—
post_admission_days	Days Since Admission	—
post_admission_hours	Hours Since Admission	—
HbA1C_NGSP	Glycated Hemoglobin Method NGSP	%
troponin	Troponin (I, T)	pg/mL
total_bilirubin	Total Bilirubin	µmol/L
direct_bilirubin	Direct Bilirubin	µmol/l
total_protein	Total Protein	g/l
albumin	Albumin	g/l
prealbumin	Prealbumin	g/l
urea	Urea	mmol/L
creatinine	Creatinine	µmol/L
glucose	Glucose	mmol/l
triglycerides	Triglycerides	mmol/l
cholesterol	Cholesterol	mmol/l
HDL	High-density Lipoprotein	mmol/l
LDL	Low-density Lipoprotein	mmol/l
atherogenic_coefficient	Atherogenic Coefficient	—
VLDL	Very Low-density Lipoprotein	mmol/l
magnesium	Magnesium	mmol/l
calcium	Calcium	mmol/l
phosphorus	Phosphorus	mmol/l
iron	Iron	µmol/l
latent_iron_binding_capacity	Latent Iron-binding Capacity of Serum	µmol/l
transferrin	Transferrin	mg/dL
potassium	Potassium	mmol/l
sodium	Sodium	mmol/L
procalcitonin	Procalcitonin	ng/mL
chlorides	Chlorides	mmol/L
LDH	Lactate Dehydrogenase	U/L
ALT	Alanine Aminotransferase	U/L
AST	Aspartate Aminotransferase	U/L
GGT	Gamma-glutamyl Transferase	U/L
alkaline_phosphatase	Alkaline Phosphatase	E/l
amylase	Amylase	Unit/l
CRP	C-reactive Protein	mg/l
uric_acid	Uric acid	µmol/L
rheumatoid_factor	Rheumatoid Factor	U/mL
cholinesterase	Cholinesterase	U/L
creatine_kinase	Creatine Kinase	U/L
anti_streptolysin_O	Antistreptolysin O	U/mL
HBDH	Hydroxybutyrate Dehydrogenase	U/L
CK_MB	Creatine Kinase MB	U/l
APO_A1	Apolipoprotein A1	g/l
APO_B	Apolipoprotein In	g/l
APO_B_APO_A1_ratio	The Ratio of ApoB/ApoA1	—
ferritin	Ferritin	µg/l

11. urine_biochemistry (1,389 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
Ca+24	Calcium in daily urine	mmol/day
Cl+24	Chlorides in daily urine	mmol/day
CREAT24	Creatinine in the daily urine	mmol/day
GLU24	Glucose in the daily urine	g/day
K+24	Potassium in daily urine	mmol/day
Mg24	Magnesium in daily urine	mmol/day
Na+24	Sodium in daily urine	mmol/day
urine_albumin_24h	Albumin in the daily urine	µg/day
24h_urine_urea	Urea in the daily urine	mmol/day
urea_in_urine	Urea in the urine	mmol/day
nitrogen_loss_per_day	Nitrogen loss per day	g/day
phosphorus24	Phosphorus in daily urine	mmol/day
urine_volume	Urine Volume in 24 hours	l/day
urine_glucose	Glucose in the urine	mmol/l
microalbumin	Microalbumin in the urine	mg/l
microalbumin24	Microalbumin in daily urine	mmol/day
total_protein_urine	Total protein in urine	g/day
PROT24	Protein in the daily urine	mg/day
urine_calcium	Calcium in the urine	mmol/L
urine_magnesium	Magnesium in the urine	mmol/L
uric_acid_urine	Uric acid in the urine	mg/l
urine_sodium	Sodium in the urine	mmol/l
urine_phosphorus	Phosphorus in the urine	mmol/l
urine_chlorides	Chlorides in the urine	mmol/l
urine_potassium	Potassium in the urine	mmol/l
urine_amylase	Amylase in the urine	U/L
urine_creatinine	Creatinine in the urine	mg/l

12. coagulation_profile (33,727 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
anti-Xa	Anti-Xa	U/ml
PT_seconds	Prothrombin Time	sec
quick_test _prothrombin_time	Quick test prothrombin time	%
prothrombin_ratio	Prothrombin ratio	—
international_normalized_ratio	International normalized ratio	—
fibrinogen_calculated	Calculated fibrinogen	g/l
fibrinogen_Clauss	Clauss fibrinogen	g/l
activated_partial_thromboplastin_time	Activated partial thromboplastin time	sec
thrombin_time	Thrombin time	sec
D-dimer	D-dimer	mg/L

13. acid_base_balance (6,185 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
A-aDO_2	Alveolar-arterial oxygen gradient	mm Hg
temperature_corrected_pH	Temperature-corrected venous pH	—
temperature_corrected_pO_2	Venous temperature-corrected pO_2	mm Hg
respiratory_index	Respiratory index	—
pH	Venous pH	—
pH_arterial	Arterial pH	—
pCO_2	pCO_2 venous	mm Hg
pCO_2_arterial	Arterial pCO_2	mm Hg
pCO_2_capillary	Capillary pCO_2	mm Hg
temperature_corrected_pCO_2	Temperature-corrected pCO_2 venous	mm Hg
pO_2	pO_2 venous	mm Hg
pO_2_arterial	Arterial pO_2	mm Hg
Na+	Sodium	mmol/L
K+	Potassium	mmol/L
Ca++	Calcium	mmol/L
Glu	Glucose	mmol/L
Hct	Hematocrit	%
Lac	Lactate	mmol/L
total_hemoglobin	Total hemoglobin	g/L
sO_2_arterial	Arterial oxygen saturation	%
BE(B)	Base excess (venous blood)	mm Hg
Beecf	Base excess (extracellular fluid)	mmol/L
paO_2/pAO_2	paO_2/pAO_2	—
$\%FiO_2$	$\%FiO_2$	%
HCO_3^-_std	Standard HCO_3^-	—
HCO_3^-	HCO_3^-	mmol/L
P/F Ratio	P/F Ratio	—
temp	Temperature	C

14. antibiotic_resistance (6,794 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
microorganism	Microorganism (pathogen)	—
biological_material	Biological material	—
antibiotics (MIC)*	Estimation of the minimum inhibitory concentration for each antibiotic	—
antibiotics (RSI)*	Evaluation of antibiotic resistance shown as R, S and I	R (Resistant) S (Sensitive) I (Intermediate

***Antibiotics:** Aztreonam, Amikacin, Amoxicillin/Clavulanate (f), Ampicillin, Gentamicin, Imipenem, Colistin, Meropenem, Nitrofurantoin, Norfloxacin, Piperacillin/Tazobactam, Trimethoprim/Sulfamethoxazole, Fosfomycin with Glucose-6-Phosphate, Cefepime, Cefixime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Ertapenem, Amoxicillin/Clavulanate, Vancomycin, Gentamicin-syn, Daptomycin, Clindamycin, Levofloxacin, Linezolid, Oxacillin, Penicillin G, Rifampin, Streptomycin-synergism, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Fusidic Acid, Quinupristin/Dalfopristin, Cefoxitin, Erythromycin, Netilmicin, Piperacillin, Cefuroxime, Cefoperazone, Cefotaxime, Amoxicillin, Moxifloxacin, Pristinamycin, Chloramphenicol, BMS-284756, Cefazolin, High-activity Mupirocin, Doxycycline, Mupirocin, Trimethoprim, Clarithromycin, Cefoperazone/Sulbactam, Ofloxacin, Ampicillin/Sulbactam (f), Ceftolozane-Tazobactam, Polymyxin B, Ceftazidime-Avibactam, Kanamycin, Kanamycin-syn, Moxalactam, Ceftaroline, Cefepime/Sulbactam.

15. bacteria_culture_test (6,101 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
biomaterial_rus	Biological material in Russian	—
biomaterial_eng	Biological material in English	—
microorganism*	Microorganism (pathogen)	CFU/ml

*__Microorganisms:__ *A. baum/haem, Chry. indolog, Chrys. meningosept, Pseud. oryzihabit, Ser. liquefaciens, Cory. striatum, Ped. pentosaceus, Staph. schleiferi, Strep. dysgal./ca, Strep. gallolytic, Strep. mitis gr., Strep. parasangui, Strep. vestibular, G. haemolysan, M. lacunata, Moraxella. sp., A. baum/calc. comp, A. baumannii, A. lwoffi/haemolyt, Acinetobac. sp., Coryn. amycolatum, Coryn. urealytic, Corynebac. sp., Corynebacterium, Bacil. cereus, P. putida, R. radiobacte, Alcalig. faecalis, S. aureus, S. epidermidi, S. haemolytic, S. capitis, S. coh-ss-coh, S. xylosus, Staphyl. carnosus, S. agalactiae, S. constellatus, Str. anginosus, Str. gordonii, Str. intermedius, Str. mitis, Str. oralis, Str. pneumoniae, Str. sanguinis, Leuconost. pseudom, Achromobacter ssp., B. cepacia CF, Bur. cepacia, Bur. gladioli, S. maltophili, Sph. paucimob, K. oxytoca, K. pne-ss-oza, K. pne-ss-pne, K. pneu/oxy, Kleb. pneumoniae, E. coli, E. coli urea+, E. cloacae, E. gergoviae, P. mirabilis, P. penn/vulg, P. penneri, P. vulgaris, S. marcescens, S. odorifera1, S. plymuthica, A. caviae, Lact. catenafor, H. alvei, P. multocida, C. violaceum, C. davisae, C. lapagei, C. neteri, C. freundii, C. koseri, K. ascorbata, Morganella morgan, P. agglomeran, P. alcalafaci, P. rettgeri, P. rustigian, P. stuartii, E. avium, E. casselifla/gall, E. faecalis, E. faecium, E. raffinosus, M. wisconsens, Klebsiella pneumo, Streptococcus con, Candida albicans, E. aerogenes, Pseudomonas aeruginosa.*

16. cerebrospinal_fluid_analysis (1,021 records)

Fields	Description	Optional
new_patient_id	Unique Patient ID	—
new_hosp_id	Unique hospitalization ID	—
post_admission_days	Days since admission	—
post_admission_hours	Hours since admission	—
lymphocytes_csf	Lymphocytes in the cerebrospinal fluid	N/in the field of view
eosinophils_csf	Eosinophils in the cerebrospinal fluid	N/in the field of view
neutrophils_csf	Neutrophils in the cerebrospinal fluid	N/in the field of view
macrophages_csf	Macrophages in the cerebrospinal fluid	N/in the field of view
monocytes_csf	Monocytes in the cerebrospinal fluid	N/in the field of view
arachnoid cells_csf	Arachnoid cells in the cerebrospinal fluid	N/in the field of view
granular spheres_csf	Granular spheres in the cerebrospinal fluid	N/in the field of view
erythrocytes_csf	Erythrocytes in the cerebrospinal fluid	N/in the field of view

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Antibacterial Effect of Nitric Oxide on the Causative Agents of Hospital-Acquired Pneumonia (Experimental Study)

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Summary

The aim of the study was to evaluate the antimicrobial effect of single and repeated nitric oxide (NO) exposure on the major pathogens of nosocomial pneumonia isolated from the sputum of cardiac surgery patients.

Materials and Methods. A 24-hour culture of microorganisms from pan-resistant isolates of *Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii,* and *Klebsiella pneumoniae* from the sputum of inpatient cardiac surgery patients with nosocomial pneumonia, as well as strains of *P. aeruginosa* and *E. coli* from the American Type Culture Collection (ATCC), were exposed to 200 ppm NO (experimental sample) or medical air (control sample) in a sealed chamber for 30 minutes. After a single or 4 repeated gas exposure at 4 h intervals, Petri dishes were placed in a thermostat at 37°C and the results were evaluated at 24 and 48 h or at 12, 24, 36 and 48 h, respectively. Grown colonies were counted using an automated colony counter and recorded as CFU/mL.

Results. No growth of clinical isolates of *P. aeruginosa* and *E. coli* was observed 24 and 48 h after a single exposure to NO. Growth of *A. baumannii* was lower compared to controls at 24 h but continued at 48 h. No effect of a single exposure to 200 ppm NO on other microorganisms was observed. After 4 exposures to NO, the growth of ATCC *E. coli* was not detected, the growth of other experimental strains was significantly lower compared to the control ($P<0.05$).

Conclusion. Our results provide a rationale for the use of multiple intermittent inhalation of 220 ppm NO for the treatment of patients with hospital-acquired bacterial pneumonia.

Keywords: nitric oxide; NO; Acinetobacter baumannii; Pseudomonas aeruginosa; Escherichia coli; Klebsiella pneumoniae; hospital-acquired pneumonia

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

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Introduction

The global death toll from infections caused by multidrug-resistant bacteria is increasing every year. Today, resistant infections cause more than 700,000 deaths per year; by 2050, this number could rise to 10 million [1, 2]. Experts around the world are warning of the high risks of the post-antibiotic era [2]. In 2017, the World Health Organization published a list of pathogens that require new antimicrobials. Carbapenem-resistant *Enterobacteriaceae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* were identified as priorities [3, 4]. These pathogens are the leading causes of nosocomial pneumonia and represent a global public health threat [5]. However, the development of new antimicrobial drugs takes decades from molecule discovery to marketing approval. Resistance to an antibiotic develops concurrently with its introduction and use in clinical practice.

Researchers around the world are working to improve the efficacy of antibacterial therapy. From this perspective, the use of nitric oxide (NO) for medical purposes is highly promising, as evidence of its antimicrobial properties has emerged in recent years. This molecule's antiviral efficacy has been described [6–18], as well as its successful use in the treatment of infected wounds [19–23] and pulmonary infection in cystic fibrosis patients [24–27]. NO has

been shown to disrupt bacterial biofilms that cause antibiotic pseudoresistance [28–35]. The ability of NO to inhibit the growth of some microorganisms *in vitro* has been studied [36–39], and exposure to NO has been shown to reduce bacterial load in a rat model of Pseudomonas aeruginosa-induced pneumonia [40]. There have been reports of the use of NO as a rescue therapy in cystic fibrosis patients, with reduced microbial load and clinical improvement [41–44]. The use of NO is especially important in the treatment of infections caused by multidrug-resistant pathogens, because its antimicrobial mechanisms differ from those of traditional antibiotics [25, 45, 46].

For successful use of NO as an antimicrobial agent in clinical practice, it is necessary to determine the dose that is effective against a particular pathogen and at the same time safe for humans, as well as the necessary duration of exposure. Authors have reported heterogeneity in the susceptibility of different strains of microorganisms to NO [36, 38, 41]. Most studies show that the NO molecule exerts its antibacterial activity at high doses (⩾160 parts per million (ppm)) [23-25, 36, 39-47]. *in vitro*, continuous exposure to high doses of NO (⩾160 ppm) for 2–10 hours, depending on the pathogen, was found to be necessary for complete bacterial killing [36, 38, 46]. Continuous exposure of the human body to NO at this concentration is considered dangerous because it leads to methemoglobinemia [39].

Most of the Russian studies on the use of high doses of NO as an antimicrobial agent dealt with its local application in the treatment of wounds [48–53].

Studies of high-dose NO inhalation are scarce in the Russian literature [54, 55]. Currently, the clinical trials registry website Clinicaltrials. gov lists 3 Russian trials on the use of inhaled NO at 200 ppm, including RECORD Pilot NCT06162455 «High-dose Inhaled NO Therapy for the Prevention of Nosocomial Pneumonia after Cardiac Surgery with Cardiopulmonary Bypass», RECORD NCT06261827 «High-dose Inhaled NO Therapy for the pREvention of nosoCOmial Pneumonia after Cardiac Surgery with caRDiopulmonary Bypass» and NO PNEUMONIA NCT06170372 «High-dose Inhalations of Nitric Oxide in the Treatment of Pneumonia».

The COVID-19 pandemic has sparked renewed interest in inhaled NO therapy. Most studies highlight the efficacy and safety of intermittent, repeated high-dose (160–200 ppm) NO therapy with an average duration of 30 minutes per inhalation in humans [8, 24, 25, 40–44, 56, 57], including pregnant women [11] and neonates [58].

The current literature on the use of inhaled NO for bacterial respiratory infections focuses exclusively on the treatment of patients with cystic fibrosis [40–44]. Therefore, investigating the possibility of using NO as an antimicrobial agent in hospital-acquired bacterial pneumonia seems very relevant.

The aim of our study was to evaluate the antimicrobial effect of NO *in vitro* after single and multiple exposure to this gas of the major pathogens of nosocomial pneumonia isolated from the sputum of cardiac surgery patients.

Materials and Methods

A 24-hour microbial cultures of panresistant strains of *P. aeruginosa*, *Escherichia coli*, *A. baumannii*, and *Klebsiella pneumoniae* from sputum of inpatient cardiac surgery patients with nosocomial pneumonia and cultures of *P. aeruginosa* and *E. coli* from the American Type Culture Collection were grown on Endo agar. A bacterial suspension was prepared and adjusted to 108 colony forming units per mL (CFU/mL) by visual comparison with the appropriate McFarland standard (0.5) [36]. 0.1 mL of the suspension was diluted with sterile 1:1000 normal saline. 2 μL of each culture suspension was inoculated into ten Petri dishes with Endo agar using a calibrated bacteriological loop and spatula according to the «spot-on-the-lawn» method. All microorganisms were provided by the Department of Laboratory Diagnostics of Tomsk Regional Clinical Hospital.

In the first part of the experiment, according to 1:1 blind randomization, 5 out of 10 Petri dishes were exposed once to 200 ppm NO for 30 min in a closed chamber (experimental group). The second 5 Petri dishes were exposed once to medical air for 30 minutes (control group). Thus, a series of 10 interventions (N=5 in the experimental group and N=5 in the control group) was performed for each microorganism. NO produced by plasma chemical synthesis was passed through an NO supply line connected to a medical air supply circuit. The supply of synthesized NO was adjusted until a target concentration of 200 ppm was reached. The resulting gas-air mixture was passed through a viral-bacterial filter into a flow chamber containing Petri dishes with the tested microorganisms. The NO concentration was continuously monitored at the inlet and outlet of the flow chamber. Petri dishes with the tested microorganisms were kept in the gas-air mixture containing 200 ppm NO for 30 minutes.

After exposure to NO or medical air, Petri dishes were placed in a thermostat at 37°C. The result was evaluated after 24 and 48 hours.

In the second part of the experiment, the effect of repeated exposure to 200 ppm NO on microorganisms was evaluated using the same methodology. For this purpose, panresistant strains of *A. baumannii* and *K. pneumoniae* isolated from the sputum of patients with hospital-acquired pneumonia, as well as cultures of *P. aeruginosa* and *E. coli* from the American Type Culture Collection, were exposed

to NO (experimental samples) or medical air (control samples) for 30 minutes 4 times with an interval of 4 hours. For each microorganism, a series of 10 sessions (*N*=5 in the experimental group and *N*=5 in the control group) was performed. After each gas exposure, the Petri dishes were placed in a thermostat at 37°C until the end of the experiment.

The experiment was performed in 2 steps.

Step 1: 24-hour bacterial culture.

1. Preparation of Endo agar. 4 g of dry nutrient medium was added to 100 cm^3 of cold distilled water, mixed thoroughly and boiled for 3–5 minutes, avoiding burning. After cooling to 40–50°C, the nutrient medium was poured into sterile tubes and arranged at an angle to obtain agar slants.

2. Preparation of 24-hour bacterial culture. Six bacterial strains were inoculated onto the slanting Endo agar, including panresistant strains of *P. aeruginosa*, *E. coli*, *A. baumannii*, and *K. pneumoniae* from sputum of patients with hospital-acquired pneumonia, as well as cultures of *P. aeruginosa* and *E. coli* from the American Type Culture Collection. The bacterial strains were grown in a thermostat at 37°C for 24 hours.

Step 2: Preparation of bacterial suspension and exposure to NO

1. Preparation of bacterial suspension. 5 mL of sterile 0.9% NaCl solution was added to the tubes containing the grown 24-hour culture of microorganisms. To obtain a suspension, the tubes were placed on the platform of a PST-60HL thermoshaker and incubated at 30°C for 10 minutes.

2. Dilution of the bacterial suspension. Using sterile pipettes, 5 ml of bacterial suspension was removed from the tubes and transferred to borosilicate glass tubes. The optical density of the suspension was measured using a Biosan Den-1B densitometer and adjusted to 0.5 McFarland's standard (to 10^8 CFU/mL) using sterile 0.9% NaCl solution.

3. Seeding on Petri dishes. 0.1 mL of the suspension was diluted 1:1000 with sterile 0.9% NaCl solution. 2 μL of the suspension was inoculated into Petri dishes with dense Endo nutrient medium using the «spot-on-the-lawn» method with a calibrated bacteriological loop and spatula.

Each microbial culture was seeded on 2 Petri dishes.

The culture plates were treated with 200 ppm NO or medical air for 30 minutes once or 4 times at 4-hour intervals. After each exposure, the plates were placed in a thermostat at 37°C according to the 4-fold protocol. Results were evaluated after 12, 24, 36, 48 hours of incubation: colonies grown were counted using a Scan 1200 Interscience automated colony counter. Results were expressed in CFU/mL. In each case, microorganism identification was performed at the end of the incubation period.

Statistical analysis of data was performed using STATISTICA 10 and IBM SPSS Statistics 26 software. Quantitative parameters were reported as median and interquartile range, *Me [Q1; Q3]*. Quantitative parameters at 4 measurement stages for related samples were compared using the Friedman test. Quantitative parameters between groups at each step of the study were compared using the Mann–Whitney test. Differences were considered statistically significant at $P<0.05$.

Results and Discussion

No growth of clinical strains of *P. aeruginosa* and *E. coli* was observed 24 and 48 hours after a single exposure. Growth of *A. baumannii* was less than the control at 24 hours, but continued at 48 hours. This finding suggests that a single exposure to 200 ppm NO for 30 minutes had a bactericidal effect against panresistant strains of *P. aeruginosa* and *E. coli* and a bacteriostatic effect against panresistant strains of *A. baumannii*. As for the other microorganisms used in the experiment, no effect of a single exposure to 200 ppm NO was found. Therefore, it was decided to repeatedly expose these strains to NO at the same concentration.

After 4 exposures to NO with an interval of 4 h, the growth of the ATCC culture of *E. coli* was not detected, while the growth of other experimental strains was significantly reduced compared to the control (Table 1).

Meanwhile, significant differences in growth compared to control were observed for *P. aeruginosa* and *E. coli* at 36 and 48 hours after the first exposure to NO, for *A. baumannii* at 12, 24, 36 and 48 hours, and for *K. pneumoniae* at 12, 36 and 48 hours (Table).

The results of repeated exposure to NO compared to the control group at 24 and 48 hours are shown in Figure.

Thus, repeated exposure to NO caused death of the ATCC strain of *E. coli*, significantly inhibited the growth of *A. baumannii*, *K. pneumoniae*, and the ATCC culture of *P. aeruginosa*.

When comparing the number of CFU/mL at 12, 24, 36 and 48 hours for each microorganism, no significant differences were found (Table).

Our key finding is the demonstration of antimicrobial activity of NO against the major pathogens of nosocomial pneumonia in cardiac surgery patients, including multidrug-resistant strains. Furthermore, a single exposure to 200 ppm NO for 30 minutes completely inhibited the growth of clinical isolates of *P. aeruginosa* and *E. coli*. The effect remained stable even after 48 hours of observation. The growth of *A. baumannii* was reduced 24 hours after a single exposure to NO compared to control samples, but this effect disappeared after 48 hours. No growth of the ATCC strain of *E. coli* was observed after repeated exposure to NO, and the growth of other microorganisms tested was less intense than in the control.

Table. Results of repeated exposure of cultures under study to NO, *Me [25; 75]*.

Microorganism	Sample	CFU/mL after the last exposure to NO				*P*-value
		After 12 hours	After 24 hours	After 36 hours	After 48 hours	
ATCC *P. aeruginosa*	NO, *N*=5	23.5 [22; 24.5]	25 [24; 26]	23.5 [22; 24]	23 [22; 24.5]	0.059
	Control, *N*=5	38.5 [36; 39.5]	40 [37; 41]	39.5 [38; 43]	40 [38.5; 41;5]	0.061
	P	0.054	0.2	**0.0025**	**0.0015**	
ATCC *E. coli*	NO, *N*=5	0	0	0	0	—
	Control, *N*=5	12 [11.5; 13]	12 [11.5; 13]	13 [12.5; 13.5]	13 [12; 14]	0.054
	P	0.051	0.051	0.0048	0.0048	
A. baumannii (panresistant strain)	NO, *N*=5	13.5 [11; 14]	15 [12; 16.5]	15 [11; 16.5]	15 [13; 15.5]	0.061
	Control, *N*=5	54.5 [51.5; 59]	55 [53; 58.5]	55 [51.5; 56]	55.5 [54.5; 58]	0.059
	P	0.004	0.0195	0.044	0.018	
K. pneumoniae (panresistant strain)	NO, *N*=5	24 [23.5; 25]	27 [25.5; 28]	26.5 [25.5; 27]	27 [23; 30]	0.062
	Control, *N*=5	53 [49.5; 54]	54 [51; 57]	53 [52.5; 57.6]	53 [52.5; 54.5]	0.058
	P	0.0013	0.51	0.022	0.039	

Note. ATCC — American Type Culture Collection. Comparison between groups was done using the Mann–Whitney test, within groups — using the Friedman test.

Hospital-acquired pneumonia is one of the most common infectious complications in cardiac surgery patients. Its incidence ranges from 2 to 36% depending on the type of surgical procedure [59–66]. The major pathogens include *Enterobacteriaceae* (including *K. pneumoniae* and *E. coli*), *A. baumanii*, and *P. aeruginosa* [67]. Hospital-acquired strains of microorganisms are characterized by high levels of antimicrobial resistance, mainly due to the production of antibiotic-destroying enzymes such as broad-spectrum beta-lactamases and carbapenemases [5, 67–74].

The main dosing regimens for antimicrobial drugs are specified in the package insert, a legally binding document. These doses are calculated based on the drug's pharmacokinetics and pharmacodynamics, as well as the microorganism's susceptibility to the antibiotic, and are valid during the period of registration and authorization for use. Over time, the microorganism's sensitivity to antimicrobial drugs naturally decreases, resulting in an increase in the minimum inhibitory concentration (MIC). To successfully treat infectious diseases caused by resistant microorganisms, increasingly higher doses of antibacterial drugs must be prescribed, and the physician must strike a balance between therapeutic and toxic doses, which is a difficult task in clinical practice. For example, patients with renal or hepatic insufficiency require dose adjustments, and achieving the required therapeutic concentration at the site of inflammation is difficult. Combinations of two, three, or more antibiotics, as well as the simultaneous administration of antibacterial drugs from the same class, are becoming more common. This is associated with a higher risk of serious side effects and significant financial costs. Various antibiotic delivery methods, such as inhaled aminoglycosides and continuous infusion, are currently being tested. Superinfection, or the activation of another etiologic strain with a different susceptibility to antibiotics, could be one of the causes of antibiotic failure. The latter is frequently associated with changes in treatment regimens and duration, resulting in complications such as antibiotic-associated diarrhea (up to ulcerative colitis) and fungal infections. The clinician is frequently confronted with a discrepancy between the pathogen's susceptibility to antibiotics reported by the microbiology laboratory and the absence of clinical effect.

Hospital-acquired pneumonia is the most common infectious complication in patients undergoing cardiac surgery [63–66]. This is due to the impact of many damaging factors on the lungs of cardiac surgery patients. After cardiac surgery, the integrity and normal excursion of the thorax are compromised, and the cough reflex is impaired. Meanwhile, the main primary component in the pathogenesis of nosocomial pneumonia is aspiration of the patient's oropharyngeal secretions colonized with opportunistic microorganisms. In cardiac surgery patients, risk factors for antibiotic resistance include prior broad-spectrum antibiotic therapy, frequent occurrence of enzyme-producing strains, and severe comorbidities.

When choosing a NO therapy regimen, it is necessary to determine the single dose, the duration of each inhalation, and the total duration of therapy that are most effective and at the same time safe for the patient. The safety of repeated NO inhalations over 30 minutes has been demonstrated in viral pneumonia [7, 8, 11]. However, the intensity and duration of the antibacterial effect of 30-minute intermittent NO exposure has not been established.

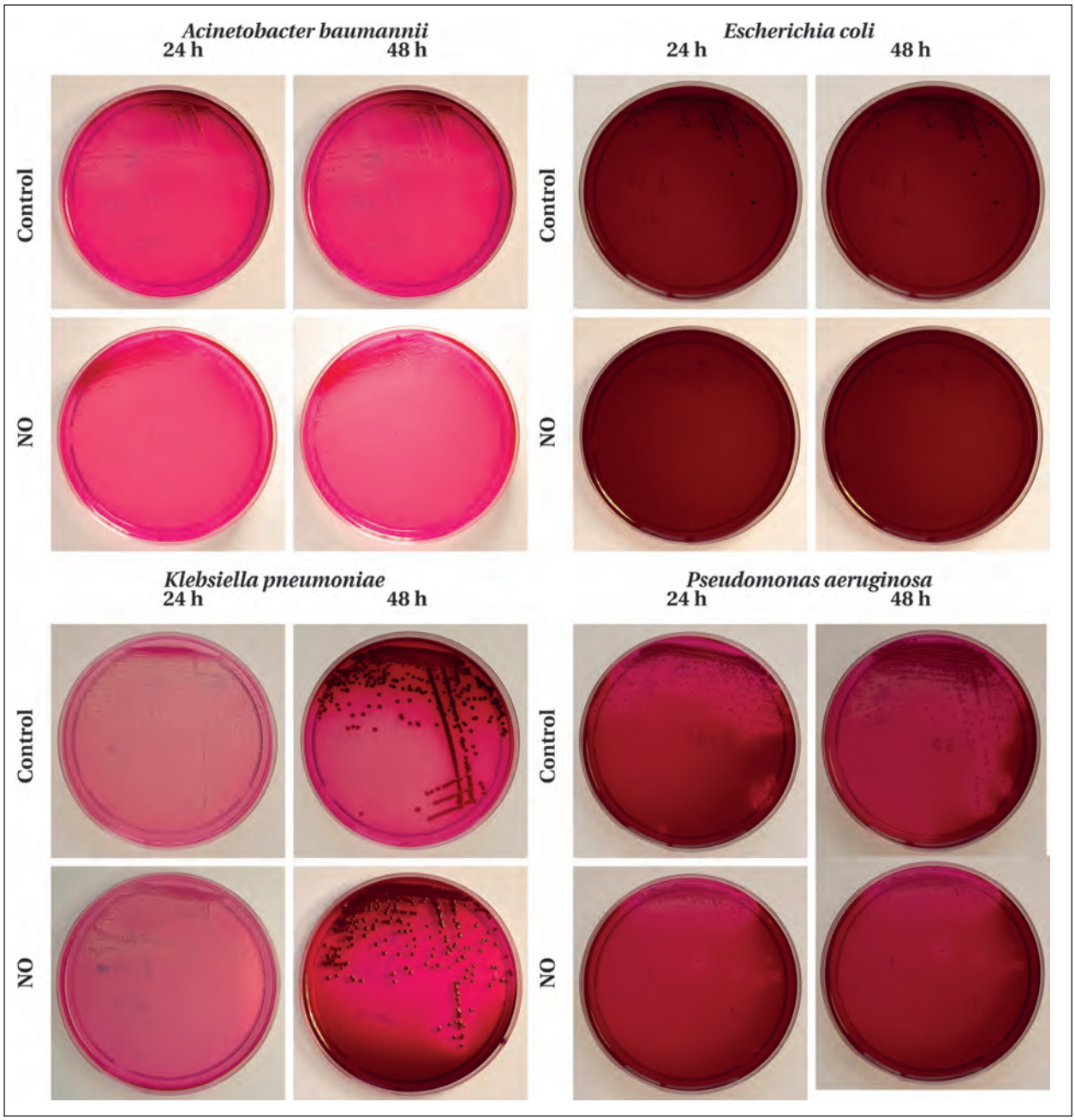

Fig. Visual evaluation of the result of repeated NO exposures to the major pathogens of hospital-acquired pneumonia *in vitro.*

The hypothesis of an antibacterial effect of repeated 30-minute exposure to 200 ppm NO on the major, including panresistant, pathogens of hospital-acquired pneumonia was tested as a clinically relevant model of NO therapy *in vitro*.

Our results show that the antimicrobial efficacy of NO persists after intermittent, repeated 30-min exposure. However, we observed a differential susceptibility of bacteria to NO action, which has been mentioned by other authors [36, 38, 41]. This is because microorganisms may have mechanisms for enzymatic inactivation of nitric oxide. One such enzyme is NO reductase, which converts NO to nitrous oxide and then to nitrogen. NO is also deactivated by oxidation by dioxygenase. In addition, some microbes can produce their own NO and use it to combat oxidative stress caused by external exposure to NO and its derivatives. As a result, microbes respond differently to NO. This has been demonstrated with common respiratory pathogens, whose susceptibility has been ranked from highest to lowest as follows *P. aeruginosa* > *Candida albicans* > *Staphylococcus aureus* > *Klebsiella pneumoniae* [75].

Our results highlight the importance of repeated NO exposure, as a significant decrease in *P. aeruginosa* and *E. coli* growth compared to the control was

only observed after 36 and 48 hours, i. e. after four NO exposures.

The use of NO is most relevant in the treatment of pneumonia caused by resistant organisms because the mechanisms of antimicrobial action of nitric oxide differ from those of traditional antibiotics [25, 45, 46]. In addition, there are observations describing the restoration of pathogen susceptibility to some antibiotics during NO therapy [43], including associations of pathogens with different antibiotic susceptibilities. NO appears to be a promising treatment for pseudoresistance caused by the ability of the pathogen to form biofilms, as it is an «anti-biofilm» agent [30]. Notably, NO therapy does not replace antibiotic therapy in pneumonia and should be used in combination with current antimicrobial agents.

Conclusion

We have demonstrated the antimicrobial effect of 200 ppm NO *in vitro* against the major pathogens of hospital-acquired pneumonia, which may provide a rationale for the use of multiple intermittent inhaled NO therapy in hospital-acquired pneumonia. This is particularly important in pneumonia caused by multidrug-resistant bacteria and in patients with risk factors for resistant microorganisms when the results of microbiological testing are pending.

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Monitoring the Immune System in Critically Ill Patients (Review)

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Summary

Most patients with critical illness, regardless of the cause, develop activation of innate and adaptive immunity. This is often a critical process leading to organ dysfunction.

The aim of the review is to systematize information on monitoring the immune system in critical illness for physicians of different specialties (anesthesiology and intensive care, surgery, general practice, obstetrics and gynecology).

The review includes information from 83 recent national and international publications (mostly from 2023), available in the public domain and found by keyword search.

We have summarized the current understanding of the relationship between infections and the human immune system, as well as the clinical application of traditional markers of immune status. We provided data on novel promising markers for the assessment of immunity in patients with various diseases.

Limitations of the studies reviewed include the need for additional large-scale clinical trials of even the most promising markers, as well as a synthesis of the evidence for their performance. In addition, immune monitoring is likely to increase the cost of patient care, necessitating the development of more affordable research methods.

Conclusion. Almost all disorders in critically ill patients are associated with changes in the immune system. Management of patients based on their immune profile requires determination of a personalized strategy for immune modulation, treatment, and prevention of infection. Advanced monitoring of immune system functions will contribute to the personalization of medicine, and the continuous development of biological technologies will allow to improve its methods.

Keywords: immune system monitoring; critical illness; biomarkers; immunity; sepsis; multiple organ failure

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Introduction

The status of the immune system is pivotal in critical illness. Whether caused by infection, trauma, or other tissue damage, most patients admitted to the intensive care unit (ICU) have an overactivation of innate and adaptive immunity. This has a vital importance and can often lead to organ dysfunction. In addition, therapeutic interventions aimed at restoring homeostasis can also alter the course of chronic diseases and promote their progression.

Secondary infections are the most common manifestation of immune system dysfunction. They are the leading cause of mortality in intensive care units worldwide and their treatment is associated with significant financial costs [1, 2].

The pathogenesis of severe infections is characterized by an uncontrolled immune response leading to excessive release of inflammatory mediators and developing immune dysfunction, which may persist for a long time even after treatment is completed [3].

In recent years, researchers have focused on infection-induced immune dysregulation due to its role in the development and prognosis of sepsis [4, 5]. Consequently, the identification of biomarkers for monitoring the immune status may provide valuable information for early diagnosis, effective prevention, and treatment of septic complications. However, despite extensive research in recent years, in-depth monitoring of the immune system in critically ill patients has not become routine.

Severe tissue and organ damage is a hallmark of critical illness. While intensivists are well-versed in monitoring the brain, heart, lungs, gastrointestinal tract, and kidneys, they are less familiar with immune monitoring and its implications for assessing immune function.

Aim of the review. To systematize information on immune system monitoring in critical illness for physicians of different specialties (anesthesiologists, intensivists, surgeons, general practitioners, obstetricians and gynecologists).

Immune Status and Infections

Immune dysfunction plays a central role in the development of sepsis complications. Pattern recognition receptors (PRRs) directly identify molecular structures on the surface of pathogens,

apoptotic host cells and damaged senescent cells. Through recognition and binding, PRRs exert non-specific anti-infective and other immunoprotective effects. Immune cells recognize pathogen-associated molecules (PAMPs) and damage-associated molecules (DAMPs) through PRRs. PAMPs are specific and highly conserved molecular structures that are unique to specific pathogens. PAMPs are essential for pathogen survival and often possess unique molecular or subcellular properties not found in host cells. Cells of the innate immune system can recognize PAMPs through PRR, distinguish between «self» and «foreign», and respond to pathogens and their products. PRR can also recognize DAMP and activate innate immunity. Binding of PRR to PAMP or DAMP leads directly to phagocytosis of pathogens by immune cells. The inflammatory response enhances the ability of the body to destroy invading pathogens. Immune cells such as natural killer cells, macrophages, dendritic cells, and parenchymal cells, both epithelial and endothelial, are involved in the early local immune response to pathogens. The interaction of PAMP and PRR activates these cells, triggering intracellular signaling pathways that involve key factors and regulate the inflammatory response [6, 7].

In most cases, the immune system effectively eliminates invading pathogens through a combination of proinflammatory responses and repair mechanisms. The proinflammatory response aims to destroy pathogens through the release of cytokines and chemokines, recruitment of phagocytes, and local activation of the complement and coagulation systems. Simultaneously, this anti-inflammatory mechanism restores homeostasis. However, in severe sepsis, the immune system cannot destroy pathogens because the dynamic balance and regulation of physiological processes are disrupted, leading to excessive inflammation and immunosuppression. The severity of immune dysfunction varies widely among individuals [8].

Sepsis manifests as a complex state of immune dysfunction, characterized by a constant release of inflammatory mediators. The characteristic inflammatory response to infection is the activation of the vascular endothelium, complement, coagulation system, and neutrophil extracellular traps. Endothelial dysfunction is present, as is the activation of platelets and B cells, both of which have closely related and cross-regulated functions. Persistent immune stimulation in severe sepsis is attributed not only to pathogen entry but also to the release of DAMPs, which activate PRRs. These PRRs often recognize PAMPs and initiate a deleterious cycle of sustained immune activation and dysfunction. Systemic activation of the innate immune system by PAMP and DAMP causes a severe and sustained inflammatory response, commonly referred to as the «cytokine storm» characterized by the excessive release of inflammatory cytokines such as IL-1, TNF, and IL-17 [9, 10].

The excessive inflammatory response leads to cell and tissue damage, molecular dysregulation, and ultimately organ dysfunction, including multiple organ failure. Sepsis patients who survive the initial hyperinflammatory phase enter the subsequent immunosuppressive phase. The relationship between hyperinflammation and immunosuppression is complex and, contrary to previous beliefs, they do not always occur sequentially. Immunosuppression can coexist with excessive inflammation, particularly in viral infections, characterized by lymphocyte depletion and reprogramming of antigen presenting cells (APCs) [11, 12].

The immunosuppression seen in sepsis is closely associated with significant depletion of key immune cell populations, including CD4+ and CD8+ T cells, dendritic cells (DCs), and B cells. The loss of lymphocytes significantly impairs the ability of the immune system to effectively fight and destroy pathogens [13, 14].

Sepsis causes delayed neutrophil apoptosis (which correlates with the severity of the disease) and a rapid increase in neutrophil levels. Although neutrophil apoptosis is delayed, accelerated apoptosis of other immune cells can compromise the host immune system by inducing dephosphorylation of epithelial caspase-8. As systemic inflammation progresses, persistent neutrophil dysfunction combined with the release of immature neutrophils eventually leads to neutrophil deficiency [15, 16].

Apoptosis-induced reduction in the number and function of DCs, which are highly efficient APCs, can lead to impaired innate and adaptive immune responses. This includes downregulation of HLA-DR expression, induction of tolerance to endotoxin, and decreased cytokine production, all of which impair the ability of APCs to stimulate lymphocytes and drive immune function. Thus, apoptosis exacerbates sepsis-induced immunosuppression of both the innate and adaptive immune systems. Therefore, exploring potential therapeutic targets to inhibit immune cell apoptosis holds great promise for reversing sepsis-induced immunosuppression [17–19].

Another phenomenon that exacerbates the patient's condition is immune cell autophagy. It is observed in almost all cell types involved in adaptive immunity, such as lymphocytes, APCs and myeloid cells. Autophagy is an important mechanism for killing intracellular bacteria that affect T and B cells. The effect of immune cell autophagy on the body is a complex process, and when immune cells can initiate programmed death, it reduces inflammation in the body. However, if autophagy is excessively enhanced, the harmful effects may outweigh the protective effects [20, 21].

Table. Immune status markers presented in the review.

Markers of immune status	
Traditional	**Promising**
Leucocyte differential	Neutrophil function markers: CD64, CD88, CD64, TREM-1, NET
C-reactive protein	HLA-DR expression
Procalcitonin	Myeloid-derived suppressor cells (MDSC)
Cytokines and chemokines	Dendritic cell assay
	Complex analysis of T and B lymphocytes
	Immune checkpoint analysis: PD-1 and PD-L1, Tim-3, CTLA-4, LAG-3, BTLA
	Analysis of changes in apoptosis and autophagy of immune cells

The ability to fight infection is also influenced by the characteristics of the patient's epigenome. Post-translational histone modifications and DNA methylation have been shown to alter the phenotype of immune cells [22, 23].

New Information About Traditional Markers of Immune Status

Inflammatory response is currently monitored using all-purpose tests that do not distinguish between the type of response or the etiology of the inflammation (Table).

The leukocyte formula is the most commonly used test to assess the immune system response and is often underestimated. After activation of the acute inflammatory response and release of adrenaline, the residence time of leukocytes in the lung or spleen decreases, contributing to a rapid increase in their number in the blood. This response is brief and nonspecific for infection, but is a sensitive marker of the inflammatory response.

In addition to absolute and relative neutrophil counts, the prognostic value of the neutrophil-to-lymphocyte ratio has been demonstrated in many studies and can be incorporated into clinical practice. In fact, neutrophil elevation is usually associated with a sharp decrease in lymphocyte count and increased mortality during critical illness [24–26]. There are potentially many reasons for such lymphopenia, such as increased apoptosis following a rapid increase in the concentration of proinflammatory cytokines, massive lymphocyte migration into tissues, decreased lymphopoiesis as an acute response to pathogenic stimuli, but the unifying pathophysiologic mechanism combining all of these factors has not yet been described.

Soluble markers such as C-reactive protein (CRP) and procalcitonin are used for bedside monitoring and clinical decision making. However, their role in the immune response is often overlooked.

CRP is mainly produced in the liver, but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes and adipocytes in response to IL-6 release. Thus, in any clinical situation with a high concentration of IL-6, there may also be a high concentration of CRP circulating in the bloodstream. CRP binds to complement molecules and, depending on the form in which it is presented (monomeric or pentameric), contributes to the opsonization of microorganisms, activation of neutrophils and monocytes, and stimulation or inhibition of the inflammatory response [27, 28].

Most infectious diseases induce a generalized immune response, so the diagnostic value of CRP is low and its use is not recommended when deciding on the use of antibiotics. On the contrary, monitoring of CRP may be useful to assess the response to pathologic agents. In patients with community-acquired or nosocomial pneumonia, a halving of the CRP level 72 hours after the start of antibiotic therapy was associated with a better prognosis and an effective response to antimicrobial therapy. On the other hand, it should be emphasized that the existing studies are very heterogeneous, some of them have methodological gaps, which does not allow drawing unequivocal conclusions on this topic [29, 30].

Procalcitonin is a molecule produced both by the parathyroid gland and by adipose tissue. In the former, its secretion is dependent on calcium and vitamin D levels, while in the latter it is released as procalcitonin in response to inflammatory stimuli such as IL-1 or IL-6. The expression of procalcitonin in adipose tissue is inhibited by IFNγ (a major cytokine involved in the antiviral response) and IL-17, which is actively released during infection. Procalcitonin should be more widely used as a test to monitor the patient's response to treatment and promote earlier discontinuation of antibiotic therapy [31, 32].

A recent meta-analysis evaluating 99 biomarkers in 15,681 patients showed that initial measurement of procalcitonin, CRP, IL-6 and sCD14 alone did not help predict mortality in critically ill patients with sepsis [33].

Thus, none of the reported soluble markers help to determine the severity of immune dysfunction and do not reflect the overall response of the host organism to infection.

In this regard, quantification of cytokines and chemokines may be more accurate in determining the nature of the immune response. Measurement of serum cytokines has provided endotypes for many manifestations of critical illness in ARDS or sepsis [34, 35].

The increasing availability of assays for the measurement of these molecules may in the future help to integrate them into clinical decision protocols

for the diagnosis of infection and immune dysfunction. Some serum cytokines are already used as markers in clinical practice. In critically ill patients, measurement of IL-6 levels was used during the pandemic and helped to determine the indication for tocilizumab [36].

Cytokine levels are thought to be associated with the development and severity of sepsis and are therefore reliable biomarkers. Examples include pro-inflammatory cytokines such as interferon-β (IFN-β) and interleukins (IL-1β, IL-3, IL-6, and IL-7) [37].

A recent meta-analysis included 145 studies reporting 26 immunological, 11 hematological, 5 inflammatory, 4 coagulation and 10 biochemical parameters. Cytokines (IL-1β, IL-1Ra, IL-2R, IL-4, IL-6, IL-8, IL-10, IL-18, TNF-α, IFN-γ, IgA, IgG) and CD4+ T/CD8, CRP, ferritin, D-dimer, serum amyloid protein A and LDH were measured. These parameters were significantly elevated in critically ill patients or in those who did not survive. In addition, patients who were not critically ill or survivors had significantly higher counts of lymphocytes, monocytes, eosinophils, CD3+ T, CD4+ T and CD8+ T cells, B cells and NK cells, as well as the lymphocyte/monocyte ratio. Disruption of innate and adaptive immune responses, as reflected by decreased levels of eosinophils, lymphocytes, monocytes, B cells, NK cells, T cells, and CD4+ and CD8+ T cell subtypes, as well as impaired blood coagulation and lung damage, were characteristic of patients with poor prognosis [38].

This is also proved by the studies of Russian researchers. Postoperative inflammatory stress response was evaluated by changes in CRP, IL-1β, IL-6. There was a correlation between the level of proinflammatory cytokines and the severity of pain [39]. Levels of systemic inflammatory response markers (IL-6, CRP) did not affect survival and length of hospital stay. Hemoperfusion in patients with severe COVID-19 provided a decrease in CRP concentration on the 1st day after administration. When started early, it promoted a significant increase in survival and shortened the duration of treatment [40]. Cytokine profiles of activated B lymphocytes and their subpopulations were determined. The results show that cytokine production by B cells is significantly dependent on the activation and differentiation of B lymphocytes [41]. A number of cytokines (IL-25, IL-33 and TSLP) are involved in the mechanism of development of allergic diseases [42]. The obtained data on changes in the levels of cytokines of the IL-10 family and IFN type III demonstrate a disturbed interaction between the systems of innate and adaptive immunity in inflammatory skin diseases with early subclinical development of severe inflammatory response [43]. The above works partially describe the alterations in immunity, but a complete picture of the immune status is still missing.

The Most Promising Markers Associated with Immune Cells

The assessment of the inflammatory response is certainly incomplete if it is based only on soluble markers and does not take into account the cellular phenotype and the expression of biomarkers in cells. These indicators reflect specific immune changes and contribute to a better characterization of the immune profile (Table).

Monitoring neutrophil function. Decreased neutrophil bactericidal activity correlates with the severity of sepsis-induced immunosuppression, particularly in patients with poor prognosis. Common markers of neutrophil function include CD64, triggering receptor expressed on myeloid cells (TREM-1), and CD88 [44, 45]. CD64 and TREM-1 are neutrophil proteins whose activation affects neutrophil function. Decreased expression of CD88 on neutrophils is closely associated with an increased incidence of subsequent secondary infections and is a strong predictor of immunosuppression [46]. Neutrophils produce extracellular traps (NETs) to capture pathogens. NETs are involved in the inflammatory response, killing and clearance of bacteria. However, their overactivation can lead to an «inflammatory storm» and damage to tissues and organs [47].

Monitoring monocyte/macrophage function. Monocytes are antigen-presenting cells that modulate adaptive and innate immunity and influence the nature of the T-cell response. Antigen presentation depends on the number of HLA-DR molecules. HLA-DR expression is a reliable marker of the antigen presenting capacity of monocytes. Poor recovery of mHLA-DR may serve as an early guide for clinicians in assessing the prognosis of patients with sepsis [48]. Reduced risk of reinfection correlates with increased mHLA-DR expression in peripheral blood monocytes of such patients [49]. Periodic monitoring of mHLA-DR expression together with CRP may help to identify patients at increased risk of sepsis in the ICU [50].

Monitoring the function of myeloid-derived suppressor cells. MDSCs were first discovered in cancer patients and mice. They significantly reduce anti-tumor immunity mediated by T and NK cells. Inflammation is a common feature of many diseases and normal physiological conditions and is the primary driver of MDSC accumulation and function. Although MDSCs are detrimental in cancer, they can be beneficial in situations where cellular immunity is overactive. Because MDSCs can be generated ex vivo, they may be employed as therapeutic agents to mitigate the damage caused by overactivated cellular immunity. MDSCs play an important role in the inhibition of innate and adaptive immune responses, including the immune responses in sepsis. MDSCs have been found to be consistently high in sepsis patients and elevated in nosocomial infections [51].

Monitoring NK cell function. The involvement of macrophages, neutrophils and DCs in the development of sepsis has been confirmed, but the role of natural killer cells (NK cells) is still unclear. On the one hand, activation of NK cells is thought to increase the risk of severe organ damage or death. However, other studies have found that activation of NK cells improves the course of sepsis [52]. The relationship between CD8+ T cells and 28-day mortality in sepsis is dependent on the number of NK cells [53]. Tim-3 expression is strongly correlated with NK cell function. Increased Tim-3 expression promotes NKT cell activation and apoptosis in the early stages of sepsis, which is associated with increased disease severity and poorer prognosis. Blocking the Tim-3/galectin-9 signaling axis with α-lactose prevents in vitro apoptosis of NKT cells isolated from sepsis patients. Disruption of Tim-3 activity protects mice against septic infection [54].

NK cells exert cytotoxic effects through the production of various cytokines, the most typical of which is IFN-γ. Serum levels of IFN-γ are an indicator of NK cell function [55].

Analysis of dendritic cells (DCs). DCs are important APCs that play a critical role in the regulation of both innate and acquired immune responses. In sepsis, the number of DCs decreases with inhibition of antigen-presenting capacity and is accompanied by abnormal cytokine secretion, resulting in impaired T lymphocyte activation. DC depletion and dysfunction are the major causes of the development of immunosuppression associated with sepsis. Based on the characteristic changes of DCs in sepsis, a novel immunomodulatory strategy targeting apoptosis, differentiation, and dysfunction of DCs has been proposed for the prevention and treatment of severe burns and trauma complicated by sepsis [56]. Activation of cannabinoid receptor 2 in acute lung injury associated with sepsis may improve disease outcome by modulating DC maturation [57]. PTEN-induced kinase 1 (dual substrate specificity phosphatase, PTEN gene product) protects against DC dysfunction during sepsis by regulating mitochondrial function control [58]. IL-3 enhances antiviral immunity by improving the recruitment and function of circulating DCs [59]. T. Zhang et al. identified novel anergic DC subtypes characterized by low major histocompatibility complex class II expression in a subset of patients studied [60]. These anergic DC subtypes were significantly more frequent in patients with sepsis.

Sepsis severity correlated with overexpression of programmed death ligand 1 on antigen-presenting cells. Combined analysis of SOFA or APACHE II scores and programmed death ligand 1 levels in monocytes and DCs may improve the quality of mortality prognosis [61]. Cell wall peptidoglycan released during bacterial replication activates human DCs as evidenced by increased expression of surface HLA-DR, CD83, T cell costimulatory molecules CD40 and CD86, and chemokine receptor CCR7. Cell wall peptidoglycan increased the production of IL-23, IL-6 and IL-1β. DCs stimulated by cell wall peptidoglycan induced differentiation of allogeneic CD4+ T cells into T helper cells producing IL-17 and IL-21 [62]. Hemorrhagic shock, through impaired DC function and maturation, inhibited cytokine production, playing an important role in immunosuppression [63].

T Lymphocytes. Monitoring cytokine production is a key measure of T cell function and differentiation. Lymphopenia is a common feature of acute inflammation [64]. Although the underlying mechanisms are not fully understood, the involvement of IL-7 is likely. IL-7 and CD127 receptor activity is associated with mortality. This activity is particularly reduced in septic shock [65].

In addition to normalizing T cell counts, maintaining a diverse repertoire of T cells and a quantitative balance between each cell phenotype is critical for full recovery of homeostasis. During post-traumatic sepsis, the T cell response «shifts» toward the TH2 phenotype, resulting in the loss of TH1. The TH17/Treg ratio has a very strong positive correlation with the SOFA score, indicating that the higher the ratio, the worse the patient's prognosis [66].

Another study looked at 2570 patients with sepsis from 25 studies. Cytokine levels were measured in the ICU before and after treatment. A meta-analysis found that a decrease in IL-6 and TNF-α levels after sepsis treatment may indicate a better prognosis and survival in patients [67]. Cytokines can play both pro- and anti-inflammatory roles. Complex interactions between cytokines, vascular cells, and immune cells lead to a «cytokine storm» and multiple organ failure, all of which contribute to the severity of the patient's condition [68].

According to the pathobiology of sepsis, biomarkers can be classified into 4 pathophysiological groups related to immune dysregulation, endothelial damage and coagulopathy, cellular damage, and organ damage. However, large and multicenter studies confirming the reliability of routine use of circulating proteins for diagnosis or prognosis in sepsis are lacking [69].

B lymphocytes. Serum IgG, IgA, and IgM concentrations directly reflect B cell status and activity [70]. Septic shock is associated with B-lymphocyte deficiency and lymphopenia. Most studies have focused on the changes in a number of immune cells during sepsis, while ignoring B cells. It turns out that B cells play a more important role in sepsis than previously thought. Both pathogen clearance and survival were reduced in B-cell-deficient mice with sepsis, whereas additional B cells improved survival in Rag1-deficient mice. Upon encountering

antigen, B cells differentiate into antibody-secreting cells and memory B cells. Most studies report a depletion of circulating B cells in patients with sepsis and a poor prognosis. Their overall depletion may be related to impaired apoptosis and maturation. Sepsis also impairs B cell function [71, 72].

In addition to decreased B cell counts, patients with sepsis develop significant B cell dysfunction. Increased expression of CD80 and CD95 on the surface of B lymphocytes is associated with an increased risk of death in patients with sepsis [73, 74].

Effects on immune checkpoints. The checkpoints are located on the surface of various cells and may reflect immune status. The most studied are the PD-1 (programmed cell death-1) protein and the programmed cell death ligand 1 (PD-L1). Studies have shown that PD-1 and PD-L1 are closely associated with cancer progression in humans and are promising therapeutic targets. In addition, the interaction between PD-1 and PD-L1 is one of the mechanisms by which human tumor cells evade the immune response. Several drugs targeting checkpoint inhibitors, including PD-1 and PD-L1, have been developed and approved for the treatment of various cancers [75]. Preclinical studies of targeted immunosuppression, particularly with immune checkpoint inhibitors, have demonstrated reversal of immune cell dysfunction and development of host resistance to infection [76]. PD-1 inhibition with nivolumab is a promising treatment option for immunosuppressed patients. It reactivates T lymphocyte function and restores immunity to fight infection [77].

Other immune «checkpoint» molecules of interest include cytotoxic T-lymphocyte antigen-4 (CTLA-4), T-cell membrane-3 (TIM-3), lymphocyte activation gene 3 (LAG-3), and B- and T-lymphocyte attenuator (BTLA) receptor.

Cytotoxic CTLA-4 is an immune control molecule expressed mainly on activated T cells and regulatory T cells (Treg), which inhibits T cell activation and regulates immune homeostasis. Based on the crucial functions of CTLA-4 in T cell biology, immunotherapies targeting CTLA-4 have been developed for the treatment of autoimmune diseases and cancer [78].

TIM-3 has been identified on the surface of T helper 1 (Th1) cells, cytotoxic lymphocytes, monocytes, macrophages, natural killer cells and dendritic cells. TIM-3 plays a key role in immune regulation. The inhibitory checkpoint TIM-3 is expressed on the cell surface in most cancers, chronic autoimmune diseases, inflammatory gastrointestinal diseases, and some viral and parasitic diseases [79].

LAG-3 (CD223) has a regulatory role similar to PD-L1 and CTLA-4, which is to inhibit immune function, cell proliferation, maintain homeostasis and cytokine production. LAG-3 is expressed on Treg cells, natural killer cells, invariant NK-T cells, activated CD4+ T helper and cytotoxic CD8+ T lymphocytes, B cells and plasmacytoid dendritic cells after antigen stimulation. High expression of LAG-3 was found in a variety of tumors. Its expression was mainly associated with poor outcomes, including tumor progression, treatment resistance and metastasis. The identified associations provide a rationale for the measurement of novel biomarkers [80].

The recent introduction of monoclonal antibodies targeting immune «checkpoints» for antitumor immunity has revolutionized the treatment of tumors. The success of therapies based on immune checkpoint blockade depends mainly on blockade of PD-1/PD-L1 and CTLA-4. However, the lack of reliable prognostic biomarkers with limited overall patient response is a major factor hindering the success of immunotherapy. BTLA may be a novel target for cancer immunotherapy. Disruption of BTLA upregulation is common and associated with poor prognosis in solid and hematological malignancies. Binding of the BTLA receptor to the herpes virus entry mediator (HVEM) ligand on the surface of T cells results in decreased cell activation, cytokine production, and proliferation [81].

In recent years, significant progress has been made in the development of approaches that focus primarily on immunotherapy, aiming to block molecules involved in immune evasion. However, there are still problems in predicting their efficacy due to the great heterogeneity of clinical responses. Thus, there is a need to develop new strategies, both in cancer and in other diseases [82].

Preclinical and early clinical studies have shown that levels of PD-1, PD-L1, CTLA-4, TIM-3, LAG-3 and BTLA cytotoxic antigens are increased on immune cells in sepsis. This is thought to be a major contributor to immune cell dysfunction. These inhibitory regulators interfere with the immune responses needed to destroy invading pathogens. Their interaction with various immune cells has been shown to inhibit innate immune functions (e. g., phagocytosis, cytokine production, and pathogen clearance) and also results in impaired T cell competence [83].

Analysis of epigenetic features [22, 23], alterations in apoptosis [17–19] and autophagy of immune cells [20, 21] are of great importance in assessing the overall picture of immune system disorders.

Currently, multiple variables are used in the ICU to assess the efficacy of hemodynamic stabilization. As a result, it is likely that more than one marker will be required to identify a reliable endotype representing immune function.

Conclusion

Almost all disorders in critically ill patients lead to alterations in immune status. Detailed mon-

itoring of immune system function is required to personalize their treatment.

Currently, there is a significant gap in our understanding of immune response trajectories and the identification of markers for effective immune monitoring. Even the most promising markers, such as monocyte HLA-DR expression, require further clinical studies.

In addition, immune monitoring is likely to increase the cost of care for patients in the ICU, necessitating the development of alternative testing methods.

Management of patients based on their immune profile requires the development of personalized strategies for immune stimulation, treatment and prevention of infection.

Successful implementation of personalized interventions requires the identification of additional biomarkers for accurate assessment of immune status. Although some biomarkers are still in the experimental stage, they hold the promise for future clinical applications. Methods for monitoring immune status are expected to improve with Advances in modern biotechnology are expected to improve the methods for immune status monitoring.

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Enolases: Limitations for Implementation in Clinical Practice (Critical Review)

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Summary

Enolases (ENOs) are involved in glycolysis, which is critical for providing energy to cells under hypoxic conditions. ENOs are attracting the attention of researchers as a potential diagnostic marker for critical conditions.

The aim of this review is to analyze the reasons limiting the clinical use of ENOs for diagnostic and prognostic purposes in critical conditions.

We selected and analyzed 87 publications in which ENOs assessment was mainly performed in patients with critical illness. Criteria for selecting relevant publications from PubMed and Elibrary were based on a presence of authors' recommendations or current guidelines on clinical use of ENOs for diagnostic or prognostic purposes.

Specific properties of human ENO isoenzymes were reviewed, clinical aspects and recommendations for their clinical use, as well as methodological and procedural errors in ENO testing were considered.

The following controversial issues were identified: the measured level of ENOs does not characterize the true enzymatic activity of their numerous molecular isoforms; identification of specific ENO isoforms using antibodies to structural subunits does not allow assessment of the true content and enzymatic activity of potentially circulating isoenzymes (e.g., gamma-gamma and alpha-gamma ENOs); the concept of cell specificity ascribed to heterodimers (in particular, gamma-alpha enolase is considered to be neuron-specific) is not supported by the results of the available studies, since this heterodimeric form of ENO is present in a variety of human tissues; some procedural issues are not taken into account (e. g., latent hemolysis, lack of standardized assessment methods, etc.).

Conclusion. The use of advanced diagnostic platforms based on the assessment of the content and enzymatic activity of each ENO isoform should provide valuable information on their specific role in the pathogenesis of diseases in the context of personalized medicine and will enable the evaluation of their diagnostic and prognostic significance, as well as the effectiveness of therapeutic interventions.

Keywords: enolases; isoenzymes; multiple molecular forms; critical conditions; clinical guidelines

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

Introduction

Various diseases and lesions leading to critical illness are characterized by severe metabolic derangements, making molecular biomarker research important for disease diagnosis, management, and prognosis [1–7].

Enolase is a promising molecular biomarker. The discovery of glycolysis, one of the primary mechanisms of glucose oxidation (the Embden–Meyerhof–Parnas pathway), prompted active research into enolases. This pathway is based on the phosphorylation of glucose, resulting in the production of pyruvate. In 1940, researchers discovered the glycolytic reactions that produce ATP and NADH, which provide energy for cellular metabolism.

Meyerhoff and Lohmann discovered enolase in the early 1930s while studying glycolysis [8]. Phosphopyruvate hydratase, also known as 2-phospho-D-glycerate hydrolase (EC 4.2.1.11) and commonly referred to as enolase, is the primary catabolic enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway for ATP production [9].

Under normal oxygen conditions, glycolytic enzymes are widely distributed throughout the cytoplasm of cells. Recent research has revealed the compartmentalization of glycolytic enzymes in response to hypoxic stress. A higher concentration of glycolytic enzymes increases the rate of glucose utilization, which boosts energy production. Disruption of compartmentalization can impair metabolism (even in neurons). Hypoxia inhibits the tricarboxylic acid cycle, making glycolysis the primary pathway for converting glucose into usable energy. However, the mechanisms that compensate for the loss of energy production caused by the inactivation of the tricarboxylic acid cycle are still poorly understood [10].

The importance of studying glycolysis for diagnostic purposes in medical practice is undeniable. In this context, enolase has attracted attention due to its potential use as a diagnostic marker in critical illness.

The aim of the review is to analyze the factors limiting clinical use of enolase for diagnostic and prognostic purposes in critical illness.

We analyzed more than 500 literature sources on enolase. 87 publications from PubMed and eLibrary databases were selected based on the authors' suggestions for the use of enolase research results for diagnostic purposes, as well as official recommendations for the use of enolases in clinical practice.

The critical review discusses the molecular forms of enolases, clinical aspects and recommendations for their clinical use, and methodological errors in the study of enolases.

Limitations of the studies included in the review: we primarily used publications on the study of enolases in critical illness.

Molecular Variants of Enolases

Enolases are highly expressed and conserved proteins with identical amino acid sequences in all organisms, from archaea to mammals [11]. According to Seki S. M. and Gaultier A., enolase activates glycolysis, which plays an important role in metabolism during inflammation and hypoxia [12].

The enolase family includes multiple molecular forms of enzymes (MMFE), a class of protein metalloenzymes with identical catalytic functions but different structures, physical and chemical properties. The diversity of MMFEs serves an important biological function. When environmental conditions change, the cell's MMFE spectrum shifts, allowing the organism to adapt more effectively. Changes in MMFEs, such as their number, activity of each form, and stability, are one of the mechanisms that control metabolic processes.

Among the MMFEs of enolases, there are genetically determined forms known as isoenzymes, which differ from each other in the primary structure of the protein. In addition, there are forms that result from epigenetic changes.

All molecular forms of enolase are found primarily in the cytosol. The short variable regions in the structure of enolases serve as contacts with cytoskeletal elements and ensure their precise localization.

Enolase isoenzymes consist of three subunits (a, β, and γ), which combine to form homo- or heterodimers.

Homodimers are composed of 2 identical subunits, including

- enolase 1 (ENO 1), or α-enolase, consisting of two α-subunits ($\alpha\alpha$)
- enolase 2 (ENO 2), or γ-enolase (also called neuron-specific enolase, NSE), consisting of two γ subunits (γγ)
- enolase 3 (ENO 3), or β-enolase, consisting of two β-subunits (ββ).

Heterodimers include $\alpha\gamma$ (NSE isoenzyme) and βγ isoforms.

ENO-S, another enolase isoenzyme, has been found in human, ram, and mouse spermatozoa. This isoenzyme is unique to spermatozoa and differs from the somatic enolases ENO 1, ENO 2, and ENO 3 in its electrophoretic mobility, high thermostability, and ability to undergo structural changes at high temperatures. It was named enolase 4 (ENO 4) [13].

A study of ENO-S expression during sperm differentiation suggested that this isoenzyme is synthesized relatively late in the haploid genome [14].

All possible dimers except βγ were detected in vivo. The molecular masses of the enolases range from 82 to 100 kDa [15].

Different genes encode the α, β and γ subunits: ENO 1 for α-enolase, ENO 2 for γ-enolase, and ENO 3 for β-enolase. Their expression changes during development, metabolic or general pathological processes [16].

Bivalent metal ions, particularly Mg^{2+}, are required for the structural stability and catalytic activity of enolase. The substrate 2-phosphoglycerate binds to the active site of enolase, which uses metallic magnesium ions as cofactors. Zn^{2+} ions have the same effect. Fluoride inhibits the enolase enzyme [17]. Molecular forms of enolases perform both enzymatic and non-enzymatic functions.

Biologic Role and Clinical Aspects of Enolases

The optimal use of a biomarker in medical practice requires high specificity and sensitivity, as well as a rapid and inexpensive method of measurement [18].

Alpha-enolase. Alpha-enolase is involved in pyruvate synthesis, acts as a plasminogen receptor, and facilitates plasmin activation and extracellular matrix degradation. Alpha-enolase is found on the surface of many cell types and contributes to tumor invasion.

α-Enolase is expressed on the cell surface of differentiating myocytes, and inhibitors of α-enolase-plasminogen binding prevent skeletal muscle regeneration [19].

The association of α-enolase with the mitochondrial membrane promotes membrane stability, while its sequestration on the cell surface is critical for plasmin-mediated pericellular proteolysis [20].

Starting from the embryonic period, α-enolase continues to be expressed in most adult tissues. Specific isoforms of enolase develop during ontogeny

in two cell types with high energy requirements: αγ- and γ-enolase are detected in neurons, αβ- and β-enolase in transverse striated muscle cells [21].

The embryonic brain has a high level of α-enolase, which decreases as neurons mature [22]. During brain differentiation, α-enolase is replaced by γ-enolase in neurons, which is a late event in nervous system development and may be a marker of neuronal maturation [23].

Overexpression of α-enolase and its post-translational modifications (acetylation, methylation, and phosphorylation) may have diagnostic and prognostic significance in many cancers. The ability of α-enolase to induce a potent specific humoral and cellular immune response makes this protein a promising target for tumor therapy [24, 25].

Overexpression of human α-enolase has been reported in a wide range of cancers and is closely associated with poor prognosis, making it a potential therapeutic target and biomarker [26].

α-Enolase is overexpressed in cutaneous melanoma cells. Overexpression of α-enolase led to increased tumor cell invasion, migration and proliferation, as well as higher pyruvate and lactate levels. Melanoma cells with disabled α-enolase showed the opposite effects. Thus, α-enolase is a potential therapeutic target in cutaneous melanoma [27].

Alpha-enolase stimulates the growth of pancreatic cancer cells. Knockout of α-enolase expression inhibited tumor cell proliferation and colony forming ability. One study found that α-enolase is an oncogenic biomarker and a potential target for immunotherapy in pancreatic cancer [28].

Another study investigated how α-enolase affects the proliferation, invasion and apoptosis of human breast cancer cells. The study suggested that α-enolase may be a therapeutic target in breast cancer [29]. Suppression of its expression reduces the proliferative activity, invasion ability, and apoptosis rate of breast cancer cells.

α-Enolase interacts with β-amyloid (Aβ) and inhibits fibril formation [30]. Proteolytic degradation of the Aβ peptide effectively destroys Aβ fibrils and reduces their cytotoxic effects. Infusing α-enolase into the brains of APP23 mice reduced Aβ deposition and cognitive impairment.

Enzymatically inactivated α-enolase was unable to inhibit the formation and destruction of Aβ fibrils. α-enolase's proteolytic activity may contribute to its cytoprotective effects and clearance of Aβ from the brain, making it a potential therapeutic target for cerebral amyloid angiopathy [30].

Several non-metabolic functions of α-enolase impact viral replication in infected cells, leading to research on its potential use as a target for treating viral diseases [31].

Gamma-enolase. Researchers and clinicians are interested in gamma-enolase (NSE), also known as αγ isoenzyme, as it is thought to be a neuron-specific marker [18].

In neurons and neuroendocrine cells, γ-enolase is associated with the plasma membrane. NSE is involved in axonal transport and its expression level varies according to the energy needs of the cell. When axons are damaged, NSE levels increase. NSE immunohistochemistry selectively labels damaged axons in the corpus callosum in diffuse axonal injury, whereas NSE is not detected in intact axons [32].

Serum NSE concentrations are higher in patients with ischemic stroke than in controls and correlate with infarct size and neurological deficits [33, 34]. Patients with a second peak of elevated serum NSE in the late phase of ischemic stroke (20%) were more likely to experience hemorrhagic transformation [35].

An NSE level of less than 2 ng/mL in the acute phase of stroke predicts a good functional outcome 12–14 days after stroke onset. NSE levels above 2.6 ng/mL are associated with a high risk of death [36]. In both young and elderly ischemic stroke patients who improved, NSE levels were either stable or decreased at the time of hospital discharge. At the same time, NSE levels increased in patients who had a poor outcome [37].

Serum NSE level is an indicator of neuronal damage and helps to predict disability and clinical outcome in patients with hypertension and ischemic stroke [38]. It has been proposed to use serum NSE levels as a biochemical marker of damage in cerebral ischemia-reperfusion after carotid endarterectomy [39].

The detection of NSE in peripheral blood may provide valuable and timely diagnostic information about stroke, especially when the time of stroke onset cannot be determined [40]. Given the correlation between serum NSE levels and cerebral infarct size, NSE may be a predictor of severe clinical manifestations of acute ischemic stroke [41]. High baseline NSE levels are associated with poor outcomes of ischemic stroke within 1 year in patients with hypertension [42].

Patients with out-of-hospital cardiac arrest who underwent body temperature management with adverse neurological outcomes were reported to have higher serum NSE concentrations with severe blood-brain barrier abnormalities than without [43].

The combination of NSE measurement and neuroimaging improves prediction of outcome after cardiac arrest with targeted body temperature management [44]. Cardiac arrest was reported in 171 of 475 patients (36%), with good neurological outcomes at 6 months for low NSE levels and poor outcomes at 6 months for high NSE levels [45].

High levels of NSE in cerebrospinal fluid were found to be a predictor of poor neurological outcome in survivors of out-of-hospital cardiac arrest [46]. In non-survivors of out-of-hospital cardiac

arrest, serum NSE levels increased during the first 72 hours, and in survivors, NSE levels decreased after 48 hours [47].

Elevated serum NSE levels have been observed in tuberculosis, chronic obstructive pulmonary disease, alveolar proteinosis, and acute respiratory distress syndrome [15]. Elevated serum NSE concentrations have been reported in patients with silicosis, which is important for diagnosis and assessment of disease severity [48].

Patients with dyspnea in SARS-CoV-2 infection have higher serum NSE levels than patients with milder disease and controls [49].

Elevated serum NSE levels have been reported in patients with small cell lung cancer [50]. NSE modulation has been shown to regulate cell proliferation, drug resistance and tumor growth [51].

NSE has been suggested as a candidate biomarker for gastric cancer prognosis [52].

However, there has been an increase in the number of publications showing results that do not support the clinical efficacy of enolase monitoring (primarily NSE).

Due to the difficulty in interpreting the results of NSE studies, a coefficient has been proposed as a method for quantitative assessment of changes in its level, eliminating the need to assess the absolute values of NSE. Coefficient values greater than 1.0 indicate an increase in NSE concentration, which could indicate progressive neuronal damage [53].

However, A. Huţanu et al. [54] questioned the use of NSE as a marker for ischemic stroke. In this study, there were no significant differences between serum NSE levels in ischemic stroke patients and controls, and a high NSE level was associated with a better outcome. In addition, NSE levels were not associated with functional outcome after three months.

A systematic review found no association between NSE levels and functional outcome or stroke severity [55]. NSE did not help discriminate between ischemic and hemorrhagic stroke [56].

There have been conflicting findings regarding the significance of NSE levels in the late phase of ischemic stroke after endovascular treatment. Blood samples were taken from 90 patients before endovascular treatment and at 2 hours, 24 hours, 48 hours, 72 hours and 3 months after treatment. Serum NSE levels remained constant throughout the study [57].

L. E. Pelinka et al. [58] found interesting results based on clinical and experimental data in a study aimed at answering the question whether NSE is an informative early marker of traumatic brain injury (TBI) and whether NSE affects ischemia/reperfusion injury of abdominal organs. It has been shown that serum NSE levels are elevated to the same extent in patients with and without polytrauma, but without TBI.

In rats, serum NSE concentrations increased more than threefold during hepatic and renal ischemia and more than two to three times after hepatic, renal, and intestinal reperfusion compared with laboratory controls. Thus, the hypothesis that NSE is an early indicator of TBI in multiple trauma was not supported [58].

Beta enolase. Beta-enolase is present in both skeletal and cardiac muscle [21, 59]. High levels of β-enolase subunits are characteristic of rapidly contracting fibers of adult muscle [60]. Beta-enolase is a marker of muscle differentiation in rhabdomyosarcoma [61].

Mutations of the *ENO3* gene, which produces the β-subunit of enolase, produce β-enolase with low stability [62]. Serum β-enolase levels are indicative of exercise-induced muscle damage in athletes [63].

Muscle β-enolase deficiency is a very rare inherited metabolic myopathy. In one study, two men, one Italian and one Turkish, whose parents were blood relatives, had several episodes of severe myalgia, cramps, generalized muscle pain, and dark urine. None of the other family members reported similar symptoms. Biochemical studies of muscle tissue showed a marked decrease in muscle β-enolase activity (20 and 10% residual activity, respectively). Molecular genetic analysis of the *ENO3* gene revealed two homozygous missense mutations [64].

Immunocytochemical analysis of transverse sections of adult mouse calf muscle allowed the expression of α- and β-subunits to be compared with the expression of myosin heavy chain isoforms. The expression of β-enolase in muscle cells is finely regulated in response to energy demands. The intensity of α-enolase expression appeared to be independent of fiber type. Confocal microscopy analysis showed that α-enolase was localized in the M-band. Most of the β-enolase was distributed throughout the sarcoplasm. Some β-enolase was localized in both the Z- and M-bands. The results of the study support the idea that isoenzymes differ in their ability to interact with other macromolecules and partition to different subcellular locations where they respond to specific functional needs [65].

ENO-S (sperm specific enolase). The ENO-S isoenzyme has been studied in spermatozoa at different stages of maturation. The electrophoresis method showed that in testicular spermatozoa ENO-S was present in 2 major bands, named S1 and S3. When ENO-S was analyzed in spermatozoa from the seminiferous tubules, bands S1, S3 and an additional band S2 were visualized, which had the same electrophoretic properties as ENO-S from ejaculated sperm.

None of the 3 ENO-S bands were detected in testicular extracts in which spermatozoa were not visualized by histological analysis. Thus, ENO-S

exists as different isoforms (electrophoretic variants) at different stages of sperm maturation. The passage through the seminiferous tubules seems to play an important role in the maturation process of ENO-S [66].

ENO-S and α-enolase. Total ENO-S and α-enolase levels of spermatozoa were measured in 30 normospermic fertile men and 20 patients with abnormospermic infertility. Total enolase level was significantly higher in total spermatozoa of patients with sperm abnormalities compared to normospermic patients. The α-enolase level in total sperm was significantly higher in abnormal than in normospermic men. The α-enolase concentration correlated positively with the percentage of immature spermatozoa with excess residual cytoplasm. The ENO-S level in total spermatozoa of normospermic patients was significantly higher than in abnormospermic patients. The studied enolase isoforms seem to reflect opposite aspects of sperm quality: α-enolase is associated with abnormal and ENO-S with normal sperm. As an additional parameter to distinguish normal from abnormal sperm, the ENO-S : α-enolase ratio was evaluated in both groups. This ratio is a marker of sperm quality and is a prognostic index of the potential of sperm to fertilize the oocyte [67].

Combining hetero- and homodimeric enolase forms. The majority of platelet and erythrocyte enolase is represented by the αγ heterodimer combined with the α-enolase homodimer [68, 69].

Two monoclonal antibodies against human and bovine γ-enolase were produced in isolated hybrid cell lines. They showed reactivity with γ and αγ isoforms of human and rat γ-enolase and with bovine γ-enolase. The antibodies did not cross-react with α- or β-subunit isoenzymes of human and rat enolase. Both antibodies partially inhibited γ- and αγ-enolase activity [70].

The distribution of 3 forms of rat enolase (α-, αγ- and γ-), including those specific to the nervous system (αγ- and γ-enolase), was determined using an enzyme-linked immunosorbent assay system. Brain and spinal cord contained more than 100 pmol/mg of α-, αγ- and γ-enolase. Organs such as lung, heart, spleen, liver and kidney had similarly high α-enolase concentrations, but αγ- and γ-enolase levels were less than 1% of their concentrations in the central nervous system. High concentrations of αγ- (greater than 10 pmol/mg) and γ-enolase (greater than 1.5 pmol/mg) were found in the rectum, bladder and uterus [71].

The gamma and αγ isoforms are classified as neuron-specific enolase (NSE). NSE is predominantly found in neurons and neuroendocrine cells and is a marker for tumors derived from these cells. It is used to monitor patients with small cell lung cancer. More recently, it has been used to monitor brain lesions. Monoclonal antibodies against γ-enolase have been produced in mice and used in the Cobas Core immunoassay system, which is a rapid, reliable and convenient test for measuring NSE levels in human serum [72].

New clinical requirements for triaging patients with chest pain are challenging the capabilities of existing cardiac markers. Serial mass measurements of creatine kinase isoforms, troponin forms, and myoglobin in emergency departments help to rapidly rule out acute myocardial infarction (AMI). However, during the first 3–4 hours after the onset of chest pain, their sensitivity is not high enough to make a significant contribution to the diagnosis of AMI. Proposed molecular markers for the early diagnosis of AMI include the αβ isoform of enolase [73].

Recommendations for Clinical Use of Enolase

The American Academy of Neurology has recommended the use of serum NSE to predict adverse outcome after global cerebral hypoperfusion in patients requiring cardiopulmonary resuscitation. However, limited availability has delayed the general use of this test for clinical decision making after global cerebral hypoperfusion [74].

A study was conducted to determine NSE levels relevant to neurological prognosis at 24, 48, and 72 hours after return of spontaneous circulation (ROSC) in a cohort of out-of-hospital cardiac arrest patients to validate previously proposed cut-off values, including ERC guidelines 2021. The results of studies using serum NSE levels to predict long-term adverse neurological outcome after out-of-hospital cardiac arrest showed higher NSE thresholds than suggested by previous publications [75].

A prospective study has also been conducted to investigate the prognostic efficacy of automated quantitative pupillometry in unconscious patients resuscitated from cardiac arrest. The validation of pupillometry combined with an NCE criterion of >60 μg/L is expected to increase the level of evidence for clinical prognosis [76].

There are no convincing data on the use of NSE in other areas of practical medicine (oncology, pediatrics).

There is no evidence to support the use of serum NSE for the diagnosis and monitoring of neuroblastoma. There is a high risk of false-positive results due to associated factors (e. g., hemolysis of the sample) and other conditions (e. g., inflammation), which significantly reduces the diagnostic value of this test [77].

Biomarkers cannot be used in standard pediatric monitoring due to a number of limitations. The main limitations are the heterogeneity of neurological complications, small cohort sizes, lack of multicenter studies, use of different neurobiomarker

evaluation methods, lack of consensus on biofluid assay validation, and lack of reference scores based on specific marker measurement techniques in biofluids [78].

Nevertheless, research into the potential applications of enolases in clinical practice continues. Mapping of specific epitopes yielded 32 most likely epitopes for enolase [79].

Several autism-specific NSE epitopes have been found in both mothers and newborns that can be used as biomarkers for the disease [80].

Methodological Flaws in Enolase Research

Despite years of research on brain injury markers, their use for stroke diagnosis, monitoring, and outcome prediction has not been translated into clinical practice [81]. Initially, these studies were based on biochemical techniques (isolation of molecular forms on columns, electrophoresis, etc.).

The enzyme-linked immunosorbent assay (ELISA) method was introduced in the 1960s and gained popularity in the 1970s and 1980s. This led to the gradual replacement of traditional biochemical research methods with ELISA, which we believe was a serious methodological error. ELISA is a very sensitive method. However, its high susceptibility to interference often results in errors that lead to erroneous conclusions and subsequent incorrect practical decisions [82].

Conventional immunoassays do not distinguish between isoenzymes [83, 84]. Analyzing the individual molecular forms of enolase has been largely abandoned. The clinical applicability of NSE quantification using conventional sandwich immunoassays is limited by lack of inter-assay agreement and falsely elevated concentrations due to hemolysis [85].

One error is the measurement of the total amount of homo- and heterodimers of the γ- and γα-isoforms of enolase.

Grouping γ-enolase and αγ-enolase as «neuron-specific enolase» is a flawed approach because they have different structures, are produced by different cells, and may have different functional properties. In this regard, the results of different ELISA-based studies of NSE in acute cerebrovascular accidents are highly inconsistent.

αγ enolase may combine the properties of α and γ subunits, but may also have properties that distinguish it from homodimeric forms of γ and α enolase. As a result, analytical methods involving sample preparation by immunoextraction of all molecular forms of NSE are being developed to study the different molecular forms of enolase [86, 84].

The primary difference between α-enolase and γ-enolase is their sensitivity to chloride ions, urea and temperature. Alpha-enolase is highly sensitive to chloride ions, urea, and temperature. In contrast, γ-enolase is more resistant to chloride-induced inactivation. Chloride ions accumulate in neurons during repetitive depolarization, making the relative insensitivity of γ-enolase to them an intriguing finding. The resistance of γ-enolase to chloride ions may have evolved to adapt to the intracellular environment of neurons, preventing inactivation of chloride-sensitive enolase when metabolic energy is most needed [84, 15].

It has been suggested to study the αγ heterodimer using antibodies against one subunit as a solid phase antibody and antibodies against the other subunit as a labeled complex [87].

Standardization of research methods is critical, including the use of reagents from the same company, the use of the same type of equipment to evaluate study results, and adherence to the chosen variant of biological sample study (sample preparation, elimination of cross-reactions, etc.).

The failure to measure the enzymatic activity of enolase was a significant methodological error. The point is that enolase is an enzymatic protein of glycolysis that produces energy under hypoxia and has cytoprotective properties.

Conclusion

The study of various molecular forms of enolases is an important trend in critical care medicine. This is because glycolysis plays a key role in the metabolism of various organs and systems during critical illness. When planning scientific research in this area, the following factors seem to be important.

First, the enzymatic activity of enolases in general and of each molecular form under investigation must be determined,

Second, antibodies against molecular forms of enolases label a specific subunit of the protein (α, γ or β). For example, antibodies to the γ subunit will detect both γ enolase and αγ enolase. Obviously, detection of both molecular forms of enolase does not allow assessment of their true levels and enzymatic activity. The solution to this problem is to isolate the enolases into single molecular forms that can be studied separately.

The notion that heterodimers are cell-specific isoenzymes is not supported by studies that have found them in cells from different organs. This certainly prevents personalized assessment of changes in molecular forms of enolases in various diseases (including critical illness).

Studies on a novel platform using methods to assess the level and enzymatic activity of each molecular form of enolase will shed light on the role of specific isoenzymes in disease pathogenesis, as well as the diagnostic and prognostic value of therapeutic

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Legislation of the Russian Federation in the Field of Protection of Genomic and Genetic Information in the Framework of Medical Diagnostics

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Summary

Objective: to formulate recommendations and proposals for improving legislation in the field of protection of genomic and genetic information, including those obtained as a result of medical diagnostics.

Materials and methods. We analyzed 18 regulatory legal acts, including 4 international, 3 acts of foreign states, 11 domestic legislation and judicial practice in the area under study. In addition, scientific works on this topic were analyzed. In the course of the study, we used the formal-logical, dogmatic, comparative method and axiological approaches.

Results. We formulated the concepts of genomic and genetic information and demonstrated differences between these concepts. We showed topical issues of information protection, identified problems in the field of legal regulation of relevant relations, formulated recommendations and proposals for improving legal regulation.

Conclusion. Based on performed research results, we recommend:

1. To incorporate in the legislation of the Russian Federation the principle that would allow the use of genetic information for further research depending on certain cases using a criteria-based approach, when such use should meet important public interests, for example, contributes to developing methods for the treatment of serious and socially significant diseases.

2. Regulate relations in the field of obtaining consent for research of biological material for scientific purposes (for example, within the framework of the Federal Law «On Personal Data»).

3. To define in Federal Law No. 86-FZ of July 5, 1996 «On State Regulation in the Field of Genetic Engineering Activities» the cases that require ethical examination in order to comply with the principle of safety of clinical trials of gene diagnostic methods, as well as in other cases.

4. Medical organizations shall ensure compliance with the rules of professional ethics in terms of data confidentiality, carry out their depersonalization, notify patients in writing about compliance with such a regime and, as a result, provide guarantees for the protection of information about patients, as well as about their relatives.

Keywords: genomic information; genetic information; data protection; legal regulation; genetic diagnosis; critical illness; personalized medicine; reanimatology

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

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Erratum

There are Erratum in the Russian version of the «General Reanimatology» journal No. 2 2024

Location	Printed	Corrected
p. 3, 65	Nueroprotection	Neuroprotection
p. 5	ley-enkephalin	leu-enkephalin
p. 19, 20	Cirs	CIRS
p. 30	GIBT	GEBT
p. 56	asphyxicial	asphyxial
p. 56	fluxmetry	flowmetry
p. 70	Akleksey	Aleksey

Instructions for Authors of the General Reanimatology Journal

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

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

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

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

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

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

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

Full version at www.reanimatology.com

Main information for the manuscript submission

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

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