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The Role of Leu-Enkephalin Synthetic Analogue in Regulation of Systemic Inflammatory Response and Prevention of ARDS in Severe Combined Injury

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Summary

The aim of the study. To study the effect of leu-enkephalin synthetic analogue on the dynamics of inflammatory response markers and organ dysfunction in patients with severe combined trauma.

Materials and methods. A prospective clinical study with historical control from two clinical centers — N. I. Pirogov State Clinical Hospital No. 1 and N.V. Sklifosovsky Clinical and Research Institute for Emergency Medicine — included men and women with severe combined trauma and the ISS scores values of 18–44, aged 18 to 70 years. Diagnostic and therapeutic approaches in all patients followed current international, national& local protocols and 2022 clinical recommendations of the Russian Society of Surgeons «Combined and multiple trauma in combination with shock (Polytrauma)». In the study group, treatment was supplemented with extended (72 hours from the admission) infusion of the test drug through a syringe dispenser following the study protocol. Effects of the test drug prolonged infusion were evaluated for the following laboratory parameters: levels of cortisol, procalcitonin, interleukin 6, NTproBNP and leukocyte count. Laboratory tests were performed at 4 time points: prior to test drug infusion, 24 hours and 72 hours after initiation of infusion, and on Day 7. The study evaluated patient's dynamics using APACHE II, SOFA and SAPS II scales and percentage of patients developing organ dysfunction (renal, respiratory, cardiovascular), rates of sepsis complications and mortality.

Results. Patients who received the test drug had significantly lower concentrations of systemic inflammatory response markers, i. e. PCT (P=0.001) and IL-6 (P=0.010) after 24 hours of follow-up vs the control group patients. The incidence of ARDS has also decreased in the study group (P=0.011 vs control). Acute kidney injury (AKI) rate was insignificantly higher in the control group (P=0.349). The duration of hospital stay in the control group was 35 (17; 51) days vs 18 (14; 30) days in the study group (P=0.140)

Conclusion. The use of leu-enkephalin synthetic analogue inhibits production of such key systemic inflammatory response markers as PCT and IL-6, and reduces PCT concentrations within 24 hours in patients with severe combined trauma. ARDS developed less frequently in the study group, but there was no significant difference in the incidence of AKI, AHF and infectious complications between the groups.

Keywords: synthetic analogue of leu-enkephalin; dalargin; systemic inflammatory response; combined trauma; ARDS; intensive care.

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

Introduction

Significant reforms in the organization of medical care for victims of severe polytrauma have resulted in the optimization of logistics for severe and very severe patients and a significant reduction in the incidence of fatal outcomes. However, trauma continues to be an enormous social and economic burden and remains one of the leading causes of morbidity and mortality among people of working age [1, 2].

Mortality from severe polytrauma (SPT) varies from 15% in developed countries to nearly 60% in developing regions, depending on the availability of emergency medical and high-tech care [3, 4]. Nearly six million people die annually from polytrauma [5].

In Russia, the mortality rate in SPT ranges from 35% to 80% and varies significantly depending on the type of injury [6]. Analysis of the structure of mortality in polytrauma shows a decreasing pro-

portion of acute blood loss with an unchanged proportion of infectious complications and multiple organ failure (MOF) syndrome [7, 8]. Systemic inflammatory response syndrome, oxidative stress and consequently endothelial dysfunction play a leading role in the pathogenesis of multiple organ failure [9].

To date, the search for drugs that prevent the development of such complications, which ultimately lead to organ dysfunction, remains a major challenge in anesthesiology and resuscitation. Recent experimental studies on the effects of a synthetic analog of leu-enkephalin (dalargin) have clearly demonstrated its anti-inflammatory and endothelial protective properties [10, 11]. The evidence obtained regarding the targeted effect of the drug on the primary pathways of MOF development was a rationale for clinical studies in patients with severe polytrauma.

The aim of the study was to investigate the effect of a synthetic analog of leu-enkephalin on changes in inflammatory response markers and organ dysfunction in patients with severe polytrauma.

Materials and Methods

We conducted a prospective clinical study with follow-up at two clinical sites: N. I. Pirogov State Clinical Hospital No. 1 of the Department of Public Health and N. V. Sklifosovsky Research Institute of Emergency Medicine of the Department of Public Health.

The study included men and women with severe polytrauma, aged 18–70 years, with ISS score of 18–44, who had no infectious diseases in the previous month and signed an informed consent to participate in the study (Fig. 1).

Exclusion criteria were:

— Infectious diseases in the previous month

— Myocardial infarction or stroke in the previous 6 months

— Transfer from another hospital 24 or more hours after multiple trauma

— Combined trauma

— Massive soft tissue crush injury

— Morbid obesity (body mass index ⩾35 kg/m²)

— Requirement for inotropic and vasopressor support as measured by the Vasoactive-Inotropic Score (VIS) [10] greater than 10 points

— Renal failure anamnesis

— A Glasgow Coma Scale level of consciousness less than 10

— Alergy anamnesis

— Drug intolerance

— Hypersensitivity to the components of the drug,

— HIV/AIDS

— Mental, physical, or other reasons that may prevent the patient from properly evaluating his or her behavior and complying with the study protocol.

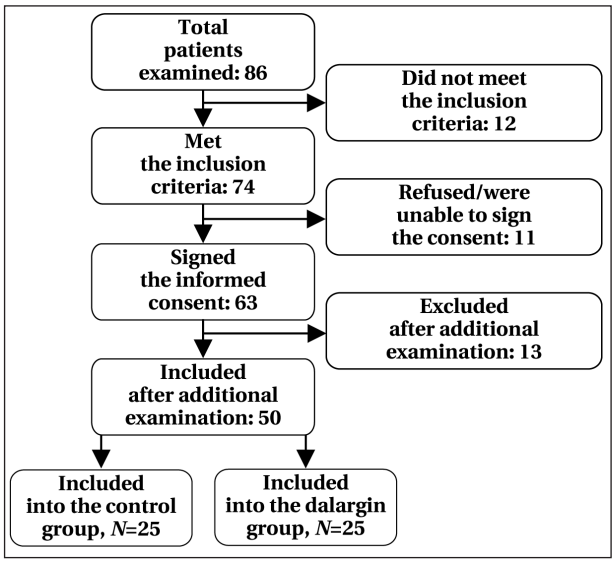

Fig. 1. Study flowchart.

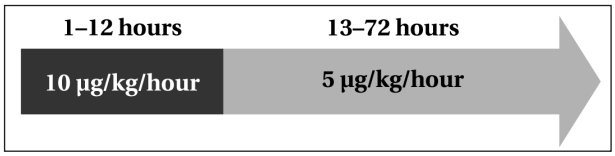

Fig. 2. The scheme of administration of the leu-enkephalin synthetic analog (dalargin) in the main group.

All patients underwent diagnostic examinations and were treated in accordance with the current international, Russian, and local protocols and clinical guidelines «Combined and Multiple Trauma with Shock (Polytrauma)» (2022) of the Russian Society of Surgeons. In the main group, treatment was supplemented with prolonged infusion of the investigational drug through a dosing device from the first hour of patient admission for 72 hours according to the study protocol (Fig. 2).

The time points for blood sampling to measure markers of the systemic inflammatory response were set at 0 (prior to study drug administration), 24, 72 h, and 7 days. Whole blood was collected from the central vein using a Vacutainer® SSTTM II Advance Vacuum Tube Blood Collection System. Serum was obtained by centrifugation of whole blood at 1500g for 15 minutes. For biomarker measurement, 500 μL of serum was aliquoted into disposable Eppendorf tubes, frozen, and stored at –20°C until the start of the study.

Serum samples (200 μL) were used to measure procalcitonin (PCT), interleukin-6 (IL-6), and cortisol concentrations using the appropriate reagent kits (Roche Diagnostics, Switzerland). Biomarkers were measured using a Cobas e411 automated electrochemiluminescence analyzer (Roche, Switzerland).

The study was conducted in accordance with the principles of the Declaration of Helsinki of the

Table 1. Comparison of sex and age characteristics and assessment scale scores.

Parameter, units	Values in groups		*P*-value
	Control, *N*=25	Dalargine, *N*=25	
Age, years (interquartile range, IQR)	34 (30–48)	35 (32–49)	0.691
Sex, male (%)	15 (60%)	14 (56%)	0.774
ISS, points (IQR)	34 (27–36)	29 (25–36)	0.697
APACHE II — day 1, score (IQR)	16 (9–23)	16 (11–23)	0.946
SAPS II — day 1, score (IQR)	30 (19–38)	35 (23–41)	0.351

World Medical Association «Ethical Principles for Scientific Medical Research Involving Human Subjects» (2013) and «Rules of Clinical Practice in the Russian Federation» (dated June 19, 2003, No. 266). The study was approved by the local ethics committee of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology on December 23, 2021, protocol No. 5/21/7. A total of 119 patients were enrolled: 57 from the dalargin treatment group and 62 from the historical control group.

Due to the systematic bias inherent in historical control studies, pseudorandomization was performed using propensity score matching (PSM). Logistic regression was used to calculate the propensity score, and the nearest neighbor method was used for matching (matching tolerance 0.008). We checked the balance of covariance in groups within strata by propensity index using standardized differences and propensity index distribution plots.

After pseudorandomization, 50 patients were included in the study, 25 in the dalargin group (main group) and 25 in the control group, including 14 men and 11 women in the main group and 15 men and 10 women in the control group (*P*=0.774), with a median age of 35 (IQR 32–49) and 34 (IQR 30–48) years, respectively (*P*=0.691). All patients with severe polytrauma were treated in the intensive care units (ICUs) of the N. V. Sklifosovsky Research Institute of Emergency Medicine and the N. I. Pirogov Hospital No. 1 in 2022–2023. The following scales were used to determine the severity of injuries and diseases ISS, APACHE II, SAPS II. The mean ISS score was 29 (IQR 25–36) and 34 (IQR 27–36) (*P*=0.697), APACHE II score was 16 (IQR 11–23) and 16 (IQR 9–23) (*P*=0.946), and SAPS II score was 35 (IQR 23–41) and 30 (IQR 19–38) (*P*=0.351) in the main and control groups, respectively (Table 1).

The most common mechanism of injury for patients was a fall from a height, followed by a train accident and then road traffic accidents (RTAs) and other causes (household trauma, industrial trauma, etc.) (Fig. 3).

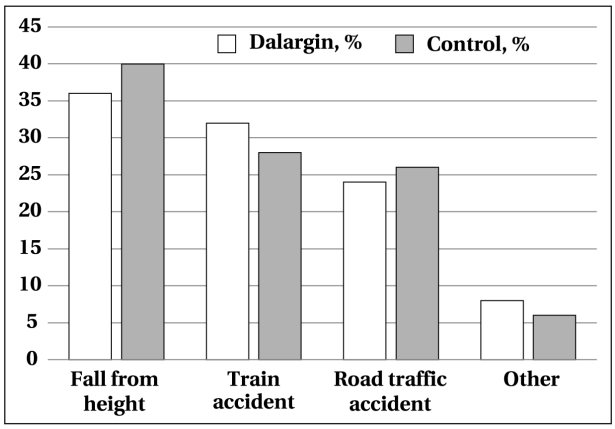

Fig. 3. Mechanisms of trauma in groups.

Statistical analysis. Data were collected and analyzed using Microsoft Office Excel 2019 software. Quantitative data were reported as *Me (Q1; Q3)*, where *Me* is the median, *Q1* is the first quartile (25th percentile), and *Q3* is the third quartile (75th percentile). Frequency variables were reported as *N* (%), where *N* is the number of cases in the group and % is the percentage of the number of cases in the group.

Normality of distribution was assessed using the Shapiro–Wilk test. The distribution of most of the quantitative unrelated variables differed significantly from the normal distribution, so differences between groups were assessed using the non-parametric Mann–Whitney U-test. Frequency variables in unrelated groups were compared using the chi-squared test or Fisher's exact test (in cases where the frequency of the outcome was less than 10%). The strength of the relationship between parameters was assessed using Spearman's rank correlation coefficient. The critical two-sided significance level p was set at 0.05. SPSS Statistics software (IBM SPSS Statistics for Windows, version 27.0.1 Armonk, NY: IBM Corp) and MedCalc® Statistical Software version 20.305 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023) were used for statistical analysis, and Microsoft Office Excel 2019 software was used to create trend graphs, dot plots, and tables.

Results

Effect of the synthetic leu-enkephalin analogue on laboratory parameters and clinical outcomes in patients with severe polytrauma. Patients receiving dalargin had significantly lower levels of systemic inflammatory response markers such as PCT (*P*=0.001) and IL-6 (*P*=0.010) after one day of observation than patients in the control group. The difference in PCT levels after one day of observation compared to

Table 2. Effect of synthetic leu-enkephalin analog on laboratory parameters in patients with severe multiple trauma.

Parameter	Values in groups		*P*-value
	Control, *N*=25	Dalargin, *N*=25	
Cortisol 0 hrs, nmol/L	778.1 (665; 821.4)	687.9 (646.7; 803)	0.256
Cortisol 24 hrs, nmol/L	501.2 (414.7; 710.5)	435.4 (296; 614.8)	0.318
Cortisol 72 hrs, nmol/L	410 (301.3; 489.7)	474.2 (316.5; 517)	0.273
Cortisol 7 days, nmol/L	683.3 (577.2; 732.7)	666 (557.6; 768)	0.982
PCT 0 hrs, ng/mL	0.13 (0.07; 0.23)	0.12 (0.07; 0.27)	0.912
PCT 24 hrs, ng/mL	1.5 (0.98; 2.77)	0.46 (0.38; 1.75)	0.001*
PCT 72 hrs, ng/mL	0.42 (0.16; 1.01)	0.19 (0.07; 0.56)	0.119
PCT 7 days, ng/mL	0.05 (0.03; 0.35)	0.07 (0.03; 0.21)	0.940
IL-6 0 hrs	188 (151.4; 215)	164.5 (123.6; 210.9)	0.322
IL-6 24 hrs	111.9 (87.7; 165.8)	75.9 (54; 114.7)	0.010*
IL-6 72 hrs	48.7 (30; 102.4)	49.8 (15.9; 79.1)	0.470
IL-6 7 days	18.5 (12.6; 47.65)	19.75 (10; 71.23)	0.689
△PCT 24 hrs — 0 hrs, ng/mL	1.23 (0.86; 1.93)	0.3 (0.1; 1.02)	<0.001*
△PCT 72 hrs — 0 hrs, ng/mL	0.29 (–0.04; 0.91)	0.04 (–0.03; 0.53)	
△PCT 72 hrs — 0 hrs, ng/mL	0.29 (–0.04; 0.91)	0.04 (–0.03; 0.53)	0.230
△PCT 7 days — 0 hrs, ng/mL	–0.04 (–0.14; 0.16)	–0.04 (–0.11; 0)	
△PCT 7 days — 0 hrs, ng/mL	–0.04 (–0.14; 0.16)	–0.04 (–0.11; 0)	0.763
△IL6 24–0 hrs	–51 (–79.2; –21.2)	–58.7 (–100.8; –20.4)	
△IL6 24 hrs — 0 hrs	–51 (–79.2; –21.2)	–58.7 (–100.8; –20.4)	0.421
△IL6 72 hrs — 0 hrs	–111.4 (–146.3; –55.5)	–88.8 (–135.6; –46.6)	
△IL6 72 hrs — 0 hrs	–111.4 (–146.3; –55.5)	–88.8 (–135.6; –46.6)	0.476
△ IL6 7 days — 0 hrs	–145.1 (–188.9; –84.4)	–110.05 (–162.2; –86)	
NTProBNP 0 hrs, pg/mL	82.85 (59.3; 187.3)	73.3 (41.5; 104.75)	0.173
NTProBNP 24 hrs, pg/mL	299.5 (123.7; 398.5)	198.7 (105.9; 318)	0.126
NTProBNP 72 hrs, pg/mL	456.35 (202.3; 723.4)	483.4 (278.9; 732.1)	0.765
NTProBNP 7 days, pg/mL	99.3 (75.2; 200.7)	112.9 (79.7; 290.2)	0.581
WBC day 1, 10^9/L	13.9 (13; 14.7)	13.9 (12.8; 14.5)	1.000
WBC day 3, 10^9/L	9.9 (8.3; 13)	9.7 (8.5; 12)	0.742
WBC day 7, 10^9/L	7 (6.5; 9)	7.2 (6.6; 7.9)	0.851
△NTProBNP 24 hrs — 0 hrs, pg/mL	153.75 (35.9; 255.1)	119.8 (42.8; 246.75)	0.859
△NTProBNP 72 hrs — 0 hrs, pg/mL	346.7 (135.3; 500.8)	365.75 (208; 576.55)	0.509
△NTProBNP 7 days — 0 hrs, pg/mL	13.4 (3.5; 120.6)	39.3 (22.1; 254.56)	0.124
△WBC day 3 — day 1, 10^9/L	–2.95 (–5.2; –1.55)	–2.75 (–4.9; –1.7)	1.000
△WBC day 7 — day 1, 10^9/L	–5.3 (–6.7; –4.6)	–6 (–7; –4.15)	0.729
△WBC day 7 — day 3, 10^9/L	–1.6 (–3; –0.9)	–2.05 (–2.7; –0.9)	0.832

Note. Here and in the Table 3: * — *P*-value<0.05.

Table 3. Effect of synthetic leu-enkephalin analog on the clinical course of severe polytrauma.

Parameter, units of measurement	Values in groups		*P*-value
	Control, *N*=25	Dalargin, *N*=25	
Men, %	15 (60%)	14 (56%)	0.774
AKI, %	4 (16%)	1 (4%)	0.349
ARDS, %	9 (36%)	1 (4%)	0.011*
AHF, %	3 (12%)	2 (8%)	0.999
Pneumonia, %	14 (56%)	9 (36%)	0.156
Meningoencephalitis, %	6 (24%)	4 (16%)	0.725
Sepsis, %	5 (20%)	3 (12%)	0.306
Death, %	6 (24%)	4 (16%)	0.725
Age, years	34 (31; 44)	35 (32; 45)	0.691
ISS, points	34 (27; 35)	29 (25; 36)	0.697
APACHE II	16 (10; 22)	16 (12; 22)	0.946
SAPS day 1, points	30 (19; 37)	35 (24; 40)	0.351
SAPS day 3, points,	19 (14; 26)	19 (15; 28)	0.662
SAPS day 7, points	10 (6; 13)	7 (4; 11)	0.08
△SAPS day 3 — day 1	–11 (–12; –5)	–10 (–14; –8)	0.613
△SAPS day 7 — day 1	–16 (–25; –12)	–23 (–26.5; –14)	0.115
△SAPS day 7 — day 3	–5.5 (–11; –4)	–11 (–15.5; –4.5)	0.178
Length of stay, days	35 (17; 51)	18 (14; 30)	0.140
Length of stay of survivors in hospital, days	38 (26; 53)	18 (14.5; 32)	0.011*
Length of stay of non-survivors in hospital, days	8.5 (3.75; 36.5)	15 (6.25; 86)	0.524
Length of stay in ICU, days	12 (5; 20)	5 (4; 14)	0.239
Length of stay of survivors in ICU, days	12 (6; 21)	5 (3; 12)	0.088
Length of stay of non-survivors in ICU, days	8.5 (3.75; 36.5)	15 (6.25; 86)	0.524
Duration of ventilation, days	2 (1; 4)	2 (0; 5)	0.702

baseline (△PCT 24h–0h) was also significantly lower in the main group ($P<0.001$) (Table 2, Fig. 4).

Regarding organ dysfunction in the early post-traumatic period, the main group showed a lower incidence of ARDS development than the control group ($P=0.011$). At the same time, acute kidney injury (AKI) was statistically insignificantly ($P=0.349$) more frequent in the control group (16%) than in the dalargin group (4%) (Table 3). The length of hospital stay was 35 (17; 51) days in the control group compared to 18 (14; 30) days in the main group ($P=0.140$) (Table 3).

Correlation analysis of the laboratory parameters. According to the classical approach to interpreting the value of Spearman's correlation coefficient (rs), some correlations were defined as strong, e. g. IL-6 24 h and PCT 24 h ($P<0.001$, $r_s=0.73$), IL-6 24 h and PCT 72 h ($P<0.001$, $r_s=0.71$), IL-6 72 h and PCT 72 h ($P<0.001$, $r_s=0.74$), WBC on day 3 and IL-6 72 h ($P<0.001$, $r_s=0.71$). These findings confirmed the strong association between IL-6 and PCT throughout the study and reflected the evolution of the inflammatory response (Fig. 5, 6).

Discussion

Multiple organ failure is a major cause of late mortality in patients with severe polytrauma [12, 13]. According to a recent meta-analysis of 17 studies with 24,267 patients, the incidence of AKI in polytrauma patients was 20.4% [14]. In a retrospective study of 2,704 polytrauma patients, 432 (16%) developed ARDS. Of these, 100 (23%) had mild, 176 (41%) moderate and 156 (36%) severe disease according to the Berlin definitions [15]. In addition, data from a recent prospective study of 297 patients with SPT and an ISS score of 29 (22–35), in which 25% were diagnosed with MOF, showed that 45% of patients developed infectious complications and hospital mortality was 15% [16].

The pathogenesis of organ dysfunction in SPT is largely determined by the duration and severity of systemic hypoperfusion of organs and tissues, which strongly influences the risk of a systemic inflammatory response in the early post-traumatic period [17, 18]. Tissue damage triggers the inflammatory response, which is mediated by «alarm signals» such as damage-associated molecular patterns (DAMPs) [19]. Further activation of neutrophils and macrophages, combined with endothelial involvement in the inflammatory cascade, contributes to organ dysfunction [11].

Several recent studies have shown a significant increase in inflammatory response markers such as PCT, IL-6 and CRP on the first day after SPT [20, 21].

Notably, a recent meta-analysis of studies on the prognostic value of PCT in patients with SPT showed that the peak PCT level on the first day after injury can be used as an early predictor of

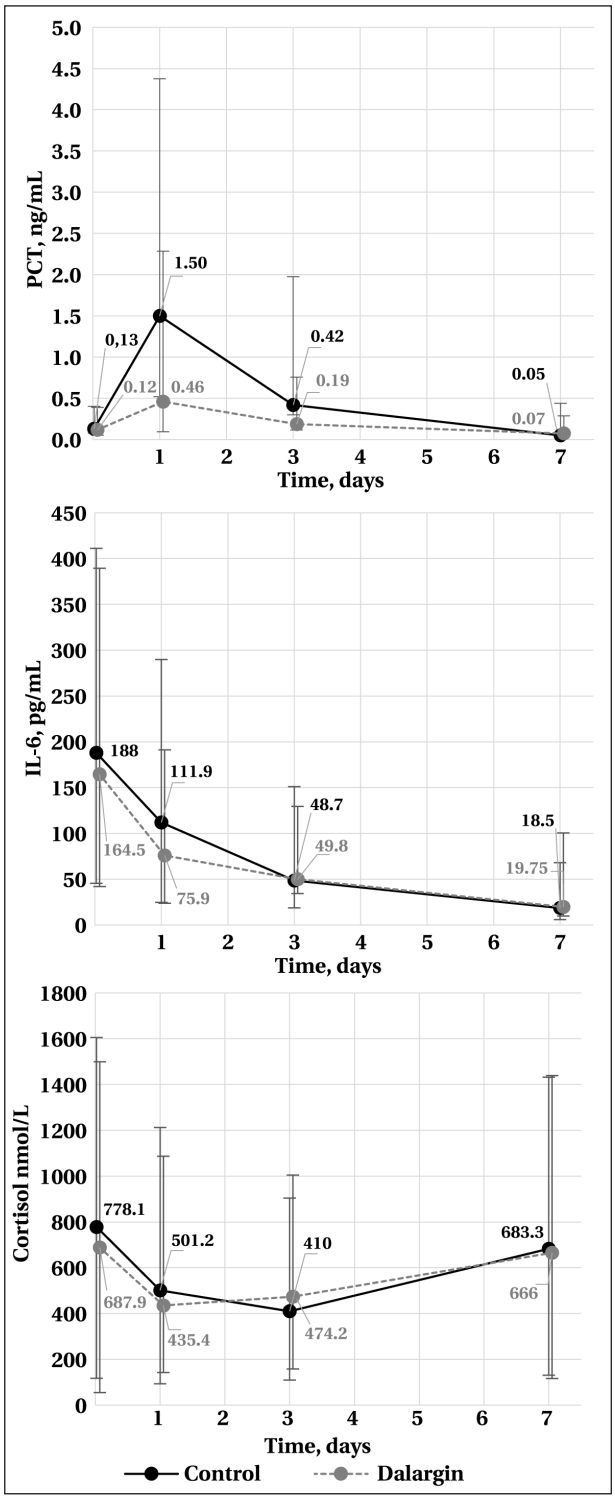

Fig. 4. Changes in laboratory parameters of the study groups.

multiple organ failure and death [22]. Another meta-analysis using data from 775 polytrauma patients found that serum IL-6 levels in the first hours after trauma were a good predictor of post-traumatic complications, especially multi-organ failure and mortality [23]. These findings highlight the importance of an excessive inflammatory response in the pathogenesis of organ dysfunction in SPT, as well as the potential for drugs to reduce its severity. At

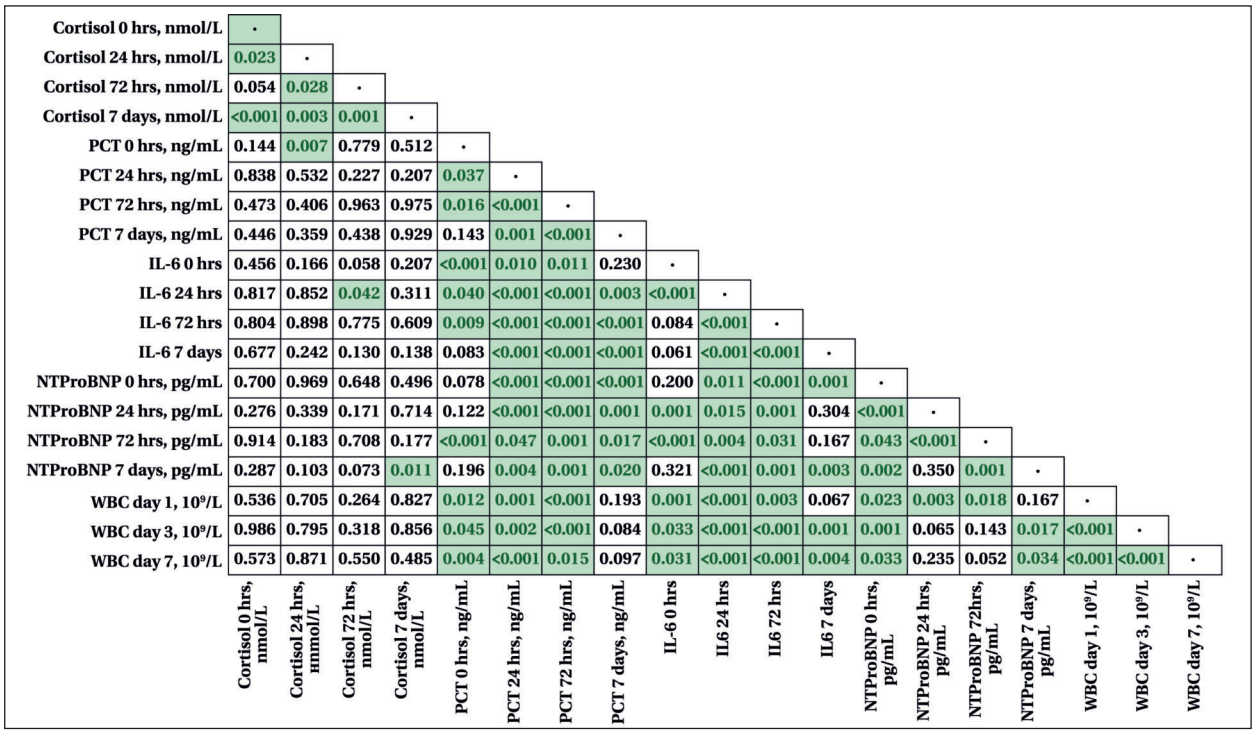

Fig. 5. Correlation analysis of laboratory parameters with *P*-values of Spearman's correlation test.
Note. Cell with green background indicates significant correlation.

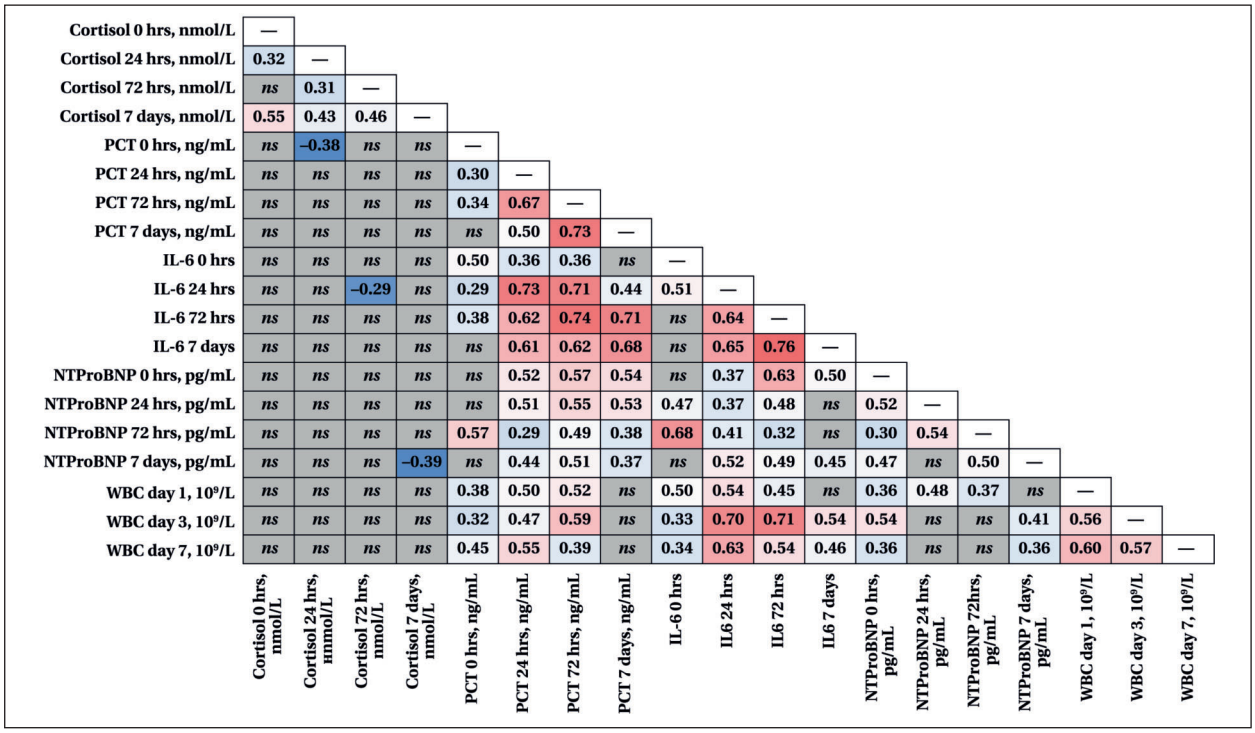

Fig. 6. Correlation analysis of laboratory parameters, Spearman's coefficient (rs) values.
Note. Warmer colors — positive correlation; colder colors — negative correlation; ns — correlation is not significant.

the same time, several studies have found that low cortisol levels on the first day after trauma are strong predictors of in-hospital mortality [24–26].

More than 35 years have passed since the introduction into clinical practice of dalargin, a synthetic analog of leu-enkephalin with μ- and δ-opioid activity, which is a hexapeptide with the amino acid sequence Tyr-D-Ala-Gly-Phe-Leu-Arg [27]. At present, dalargin is approved for clinical use only for the treatment of duodenal and gastric ulcers and acute pancreatitis as part of a comprehensive therapy.

To date, several in vitro studies have demonstrated a protective effect of dalargin on endothelium [28] and an anti-inflammatory effect on LPS and formyl peptide (fMLP)-activated neutrophils [29]. Recent *in vivo* studies also confirmed the anti-inflammatory properties of this synthetic analog of leu-enkephalin in a murine model of acute respiratory distress syndrome, as indicated by a decrease in blood IL-6 and mortality [30, 31].

Our study confirmed that dalargin can be used in patients with SPT to reduce the severity of the inflammatory response, as convincingly demonstrated by a significant decrease in IL-6 and PCT levels on the first day after injury. We also obtained encouraging data on the reduction of the incidence of ARDS in the early post-traumatic period. At the same time, dalargin infusion had no effect on the severity of the sympathetic response to severe traumatic injury.

It is important to note that only a few small RCTs have been conducted to date, one of which showed an improvement in clinical outcome with the use of dalargin in patients with moderate to severe acute respiratory distress syndrome with underlying severe and critical COVID-19 [32], and another study demonstrated a significant reduction in oxidative stress markers in patients with SPT [33].

The influence of dalargin on changes in the marker of myocardial damage, NT-ProBNP, also remains somewhat «terra incognita». Undoubtedly, there is a strong correlation between this protein level and inflammatory markers (IL-6 and PCT), but most likely its increase is not associated with disorders that develop due to polytrauma. Given the important role of both acute and delayed myocardial injury in worsening the prognosis of multiple organ failure, evaluation of the effect of dalargin on natriuretic peptide levels and the underlying processes is an extremely promising and important area for further study.

Study limitations. The sample size of 50 patients with SPT from two Moscow hospitals was small (only 25 observations in each group), making it insufficient to analyze important parameters such as treatment outcomes and drug side effects. We also did not examine the long-term effects of trauma and degree of disability after discharge from the hospital.

Conclusion

The use of a synthetic leu-enkephalin reduced the production of key systemic inflammatory response markers such as PCT and IL-6, resulting in lower PCT levels on the first day of treatment in patients with severe polytrauma. ARDS was less common in the main group and there were no significant differences in the rates of AKI, AHF or infectious complications between the groups.

The results of this study and cumulative experience in exploring the organ protective properties of the synthetic analog of leu-enkephalin warrant conducting a large multicenter RCT of the drug effects in SPT patients.

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Prognostic Markers of Acute Suppurative Lung Disease

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Summary

The mortality rate among patients with acute suppurative lung diseases (ASLD) in the ICU reaches 30%. Early, pathogenetically relevant biomarkers are needed to ensure personification and better efficacy of ASLD treatment. Numeric variations in the counts of immune system cells in patient's blood can be viewed as such candidate biomarkers.

The aim of the study. Identification of potential markers predicting ASLD outcome after community-acquired pneumonia and COVID-19.

Materials and methods. The study included 216 in-hospital patients aged 18–87 with ASLD after community-acquired pneumonia with (*N*=81) and without (*N*=135) COVID-19 history.

Results. Patients survival after COVID-19 was linked to lymphocyte count on Day 1 of hospital stay (hazard ratio, HR=5.9 95%CI 0.9–37.4; *P*=0.0188, log-rank test). In patients who had not have COVID-19, a difference in survival was associated with lymphocyte (HR=2.9 95%CI 1.0–8.4; *P*=0.0184, log-rank test; *N*=135), and monocyte counts (HR=2.7 95% CI 0.8–9.5; *P*=0.0196, log-rank test) on Day 1 of hospital stay. Patients' survival after COVID-19 infection depended on SII (systemic immune-inflammation index. HR=9.3 95%CI 1.7–49.8; *P*=0.0124, log-rank test; *N*=81, SIRI (systemic inflammatory response index, HR=7.2 95%CI 1.4–36.6; *P*=0.0339, log-rank test; *N*=81) and NLR (neutrophil-to-lymphocyte ratio, HR=9.6 95%CI 1.8–52.0; *P*=0.0108; log-rank test; *N*=81) values on Day 1 of hospital stay. In patients who did not have COVID-19 SII values had no influence on survival.

Conclusion. The lymphocyte count makes it possible to predict outcomes of pleural empyema, regardless of patient's history of COVID-19, i. e. a decrease in the lymphocyte count below 1.2×10^9 in 1 L is associated with fatal outcome. Monocyte count carries prognostic information for cases of pleural empyema without previous COVID-19 infection. As for the relative indicators, SIRI, SII and NLR values measured on Day 1 in the hospital were predictors of ASLD outcome only in patients after COVID-19 infection, i. e., higher values were associated with increased risk of death, with NLR index being the most informative. Overall severity of illness above 10 scores by CIRS was associated with an unfavorable ASLD outcome, regardless of patient's history of COVID-19.

Keywords: acute suppurative lung diseases; COVID-19; pleural empyema; lung abscess; immune system cells; SIRI; SII; NLR

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

Introduction

Suppurative diseases of the lung and pleura (SDLP) are characterized by inflammatory infiltration and subsequent destruction of lung tissue due to activities of infectious agents [1]. Despite improvements in treatment, there has been an increasing trend in morbidity, resulting in high mortality rates of 5–30% [2–4]. Several factors, including age, nutritional status, comorbidities, immunity, timely antibiotic therapy, and supportive care, play an important role in determining the patient's condition [5].

Pleural empyema is a common manifestation of SDLP. It involves the accumulation of pus or fluid with evidence of infection in the pleural cavity, with inflammatory involvement of both the parietal and visceral pleura and secondary compression of lung tissue. The main etiology of pleural empyema (in 60% of cases) is community-acquired pneumonia. Parapneumonic effusion and purulent destructive processes in lung tissue are the main causes of pleural empyema. In some patients, pleural empyema without fistula results from parapneumonic effusion, whereas in other patients with underlying lung destruction, a fistula may develop, worsening the course of SDLP [1, 6]. Bronchopleural fistula is characterized by an abnormal channel lined with bronchial epithelium, forming a persistent connection between the bronchial tree and the pleural cavity. It is a serious complication of SDLP and surgical interventions, leading to persistent lung collapse and chronic inflammation in the pleural cavity [7]. Patients with bronchopleural fistula are at increased risk of sepsis, septic shock and multiple organ failure (MOF), which significantly worsens their prognosis [8]. Although modern diagnostic methods for SDLP are well known, predictors of its course and outcome have not been established.

It seems promising to investigate two groups of indicators for this purpose: 1) cellular biomarkers, such as neutrophils, lymphocytes, and monocytes; and 2) relative indices, including NLR (Neutrophil to Lymphocyte Ratio), SIRI (Systemic Inflammatory Response Index, calculated by multiplying NLR by monocyte count), and SII (Systemic Immune Inflammation Index, calculated by multiplying NLR by platelet count).

Neutrophils are phagocytic leukocytes that constitute the «first line» of the host immune response to invading pathogens through a variety of mechanisms, including chemotaxis, phagocytosis, reactive oxygen species (ROS) and granule release, cytokine production and release, and neutrophil extracellular trap (NET) formation [10, 11]. Neutrophils also play an important regulatory role in adaptive immunity: they recruit, activate and program other immune cells (B cells, NK cells, CD4, CD8 and $\delta\gamma$ T cells) and secrete a variety of pro-inflammatory and immunomodulatory cytokines and chemokines [12, 13]. Lymphocytes are the cells of the immune system that provide adaptive immunity, the main components of which are T and B cells. T cells ensure the full development of cellular and humoral adaptive immunity through intercellular interactions with other cells, while B cells are responsible for the direct production of antibodies, which are essential for humoral immunity [14].

CD4+ and CD8+ T lymphocytes are critical for defense against sepsis [15]. Systemic inflammation results in a marked suppression of cellular immunity, causing a reduction in CD3+ T cells, CD4+ T cells, CD8+ T cells, and NK cells [16, 17]. Monocytes are short-lived circulating cells that participate in inflammation both by direct action, releasing cytokines, and by differentiation into dendritic cells and macrophages [18, 19].

The neutrophil-to-lymphocyte ratio (NLR) is a biomarker that assesses the systemic inflammatory response and predicts outcomes in several diseases, including cerebrovascular events [20], cardiovascular disease [21], bacterial and fungal infections and sepsis, community-acquired pneumonia, SARS-CoV-2 infection [22], metabolic syndrome [23], rheumatoid arthritis [24], several cancers [25, 26], decompensated liver cirrhosis [27], and severe trauma [28]. NLR is calculated by dividing the absolute number of neutrophils by lymphocytes per unit volume [29], and is readily available and convenient for use in clinical practice [30]. The NLR is thought to reflect the balance between innate (neutrophils) and adaptive (lymphocytes) immune responses [31]. SIRI and SII have been used as prognostic markers in cancer, stroke, and cardiovascular disease [32–34].

However, the prognostic value of potential markers in SDLP is not well established. Therefore, the aim of this study was to identify potential markers for the outcome of SDLP in survivors of community-acquired pneumonia and COVID-19.

Since COVID-19 is characterized by a wide range and variability of possible clinical manifestations and can lead to the development of pulmonary complications, including SDLP [35–39], it was of interest to evaluate the prognostic value of potential markers separately in groups of patients with SDLP who had undergone COVID-19 and those who had not.

Materials and Methods

We conducted an uncontrolled, prospective, observational, randomized trial that was approved by the Ethics Committee of the V. A. Negovsky Research Institute of General Reanimatology, protocol No. 2/22/1 dated July 26, 2022. The study recruited participants between November 2021 and August 2023.

Based on our initial data, the mortality rate for pleural empyema is estimated to be approximately 10 percent. We used this information to determine the required sample size. The sample size, calculated using the formula $n=(t2*P*Q)/\triangle 2$, where t is the critical value of Student's criterion (1.96 at a significance level of 0.05), $\triangle$ is the maximum allowable error (5%), P is the proportion of cases in which the studied parameter occurs (90), and Q is the proportion of cases in which the studied parameter does not occur (10), was determined to be 138.

Patients and Treatment. The study included 216 patients with SDLP that developed as a result of community-acquired pneumonia in the previous 30 days, including 81 patients who underwent COVID-19 and 135 who did not undergo COVID (Fig. 1). The total cohort included the group of patients with pleural empyema (PE) without fistula (due to parapneumonic effusion) (*N*=127) and the group of patients with pleural empyema with fistula

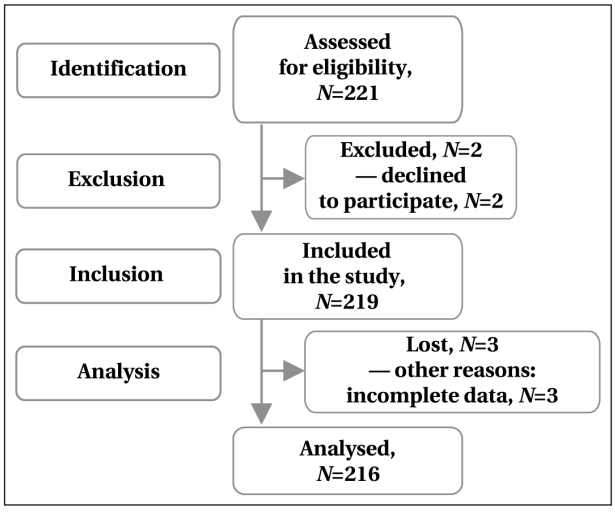

Fig. 1. Study flowchart.

(PEF) due to complicated parapneumonic effusion with bacterial contamination, lung abscess, or destructive pneumonia (*N*=89).

The diagnosis of SDLP was based on the computed tomography findings [40]. The conclusion that the patient was infected with SARS-CoV-2 was based on the results of PCR diagnostics, regardless of the date.

COVID-19 was treated according to the current version of the «Provisional guidelines for the prevention, diagnosis, and treatment of COVID-19». Pleural drainage or videothoracoscopic pleural drainage was performed in all the patients. NLR, SII, and SIRI values were calculated. Data were retrieved from the EMIAS database. Missing or incomplete data were excluded from analysis.

General criteria for inclusion in the study:

— Presence of SDLP (pleural empyema without fistula, pleural empyema with fistula, lung abscess) in a patient who had a community-acquired bacterial or viral lung infection in the previous 30 days or confirmed by COVID-19 PCR data at different times before hospitalization;

— Age 18 years or older;

— Written informed consent to participate in the study;

— The patient's ability to cooperate adequately for an extended period of time during the clinical trial.

Study exclusion criteria were:

— Refusal of further observation by the patient and/or his/her legal representative;

— Evidence of cancer or tuberculosis.

On admission, the presence or absence of diabetes mellitus was noted and the patients were assessed using SOFA, APACHE II, Charlson, CIRS (Cumulative Index Rating Scale), and RAPID scales (Table 1). Blood analysis was performed using a Sysmex XN-1000 automated hematology analyzer.

When comparing patients in the PE and PEF groups, we found that males had a higher incidence of empyema with fistula than females (*P*=0.023, FEM, OR=2.09, 95%CI: 1.12–3.9). There were no differences in age (*P*=0.394), frequency of DM (*P*=0.386), Charlson (*P*=0.694), CIRS (*P*=0.292), SOFA (*P*=0.483), APACHE-2 (*P*=0.173), or RAPID (*P*=0.274) scores on admission.

The normality of distribution of the quantitative variables was assessed using the Shapiro–Wilk test. Parameters with a normal distribution were reported as the arithmetic mean *(Me)*, standard deviation *(SD)*, and 95% confidence intervals (95% CI). Quantitative data with non-normal distribution were reported as median *(Me)* and lower and upper quartiles *(Q1–Q3)*. Variables with a normal distribution were compared between groups using the Student's t-test if the variance was equal. If the distribution pattern differed from normal, the Mann–Whitney *U*-test was used. Categorical data were expressed as absolute values and percentages. Percentages in contingency table analyses were compared using the χ^2 criterion with Yates' correction for sampling continuity and Fisher's exact method (FEM). The odds ratio with 95% confidence interval (95% CI) was used as a quantitative measure of the effect when comparing relative rates. A log-rank test was used for the Kaplan–Meier survival analysis. The results were presented as hazard ratios (HR) with 95% confidence intervals (CI). The ROC curve method was used to predict the probability of an adverse outcome (mortality). Differences were considered statistically significant at $P<0.05$. Statistical analysis was performed using MedCalc version 11.6 and SigmaStat version 3.5.

Results

Age and sex did not affect the outcomes of SDLP (Fig. 2, *b*). When analyzed separately in patients with or without COVID-19, no difference in survival was found based on sex and age (Fig. 2, *d*, *e*, *g*, *h*). An increase in SOFA score on day 1 of hospitalization was associated with a poor outcome in the entire patient cohort (Fig. 2, *c*). In patients who did not have PCR-proven COVID-19, a similar association persisted (Fig. 2, *i*); however, in patients who had COVID-19, no differences in SOFA-dependent survival with a SOFA score on day 1 of hospitalization were revealed (Fig. 2, *f*).

We found an association between comorbidity severity and mortality (Fig. 3). An increase in the CIRS comorbidity scale score above 10 was associated with an adverse outcome both in the entire patient cohort (Fig. 3, *a*) and in patients who survived COVID-19 (Fig. 3 *d*) or did not have COVID-19 (Fig. 3, *g*). A Charlson comorbidity index score

Table 1. Characteristics of patients included in the study.

Parameter	Value
Men, *N* (%)	151 (70)
Women, *N* (%)	65 (30)
Age, *Me (IQR)*	54 (41–66)
SOFA score on admission, *Me (IQR)*	2 (2–2)
APACHE II score on admission, *Me (IQR)*	5 (3–8)
Diabetes mellitus, *N* (%)	33 (15)
Charlson comorbidity index, *Me (IQR)*	2 (1–4)
CIRS comorbidity score, *Me (IQR)*	10 (7–13)
RAPID pleural infection assessment [41], *Me (IQR)*	1 (1–2)

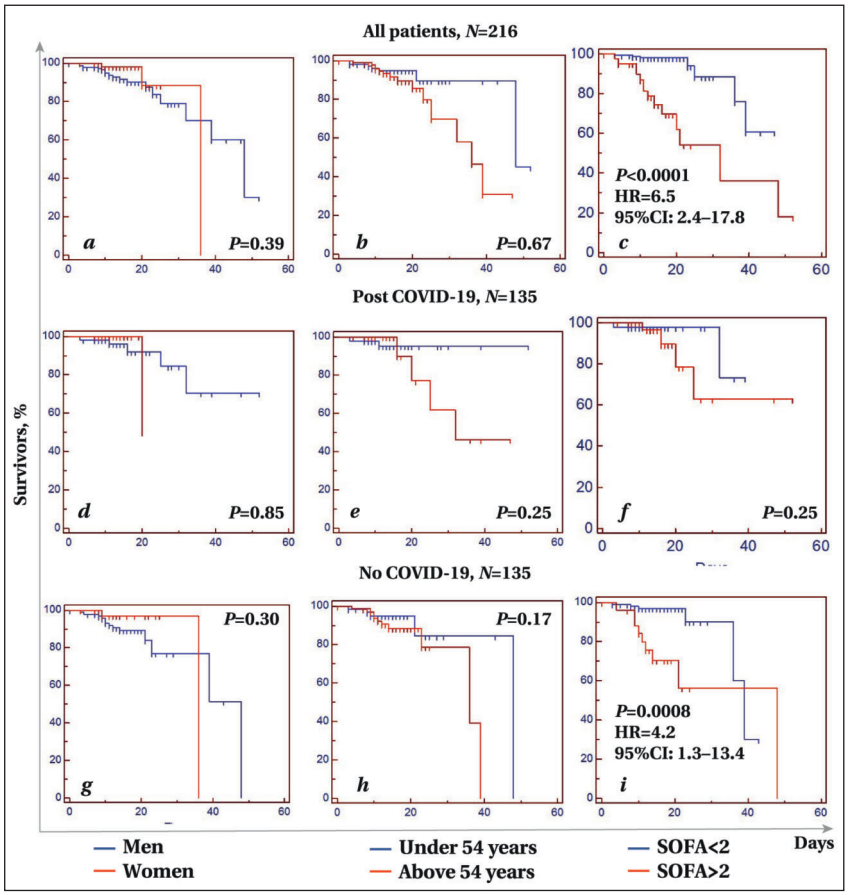

Fig. 2. Prognostic significance of sex, age, and SOFA score on day 1 of hospitalization for outcome in pleural empyema regardless of fistula formation (Log-rank test.).

Total mortality was 21 cases (9.7%). The causes of death included sepsis (14 cases, 66.6%), pulmonary hemorrhage (4 cases, 19.1%), and pulmonary embolism (3 cases, 14.3%). The total length of hospital stay was 14 (11–18) days. The detailed analysis of causes of death and length of hospital stay in the PE and PEF groups with/without COVID-19 history is shown in Table 2.

The results of linear regression analysis of demographics, scores, and cellular parameters of the total patient cohort are presented in Table 3. SOFA, RAPID, and Charlson index scores correlated with patient age. CIRS, RAPID and Charlson index scores correlated with sex. In women, the mean Charlson index score was higher and the CIRS and RAPID scores were lower than in men. Increased SOFA scores correlated with increased CIRS scores, adverse outcomes, increased neutrophil count, and decreased monocyte count. CIRS score predicted outcome. Although the CIRS comorbidity scale and Charlson index scores correlated, the Charlson index score did not influence the outcome of pleural empyema. Increased monocyte counts correlated with increased neutrophil and lymphocyte counts; however, only neutrophil and monocyte counts significantly predicted outcome (Table 3).

greater than 2 also predicted mortality in the entire patient cohort (Fig. 3, *c*), but no such association was found in patients who survived or did not have COVID-19 (Fig. 3 *e*, *h*). The use of the RAPID scale to assess patient functional status also contributed to the prediction of outcome. RAPID index scores >2 represented unfavorable prognostic markers in the total patient cohort (Fig. 3, *c*). However, in patients who had survived COVID-19, the RAPID score had no prognostic significance (Fig. 3, *f*), and in patients who did not have COVID-19, the association of poor survival with increased RAPID score values persisted (Fig. 3, *i*).

The results of linear regression analysis of demographics, scores, and cellular parameters as a function of history of COVID-19 are shown in Table 4. The NLR value correlated with SOFA, CIRS, RAPID scores, Charlson index, and outcome in the entire patient cohort and in patients without a history of COVID-19.

Table 2. Causes of death and length of stay in the PE and PEF groups.

Parameter	Values in groups					
	Pulmonary empyema (PE)			Pulmonary empyema with fistula (PEF)		
	Total cohort	Post COVID-19	No COVID-19 history	Total cohort	Post COVID-19	No COVID-19 history
Mortality, *N* (%)	2 (1.5)	1 (2.0)	1 (1.2)	19 (21.3)	5 (15.6)	14 (24.5)
Causes:						
sepsis	2 (100)	1 (100)	1 (100)	12 (63.2)	4 (80)	8 (57.2)
pulmonary hemorrhage	—	—	—	4 (21)	1 (20)	3 (21.4)
pulmonary embolism	—	—	—	3 (15.8)	—	3 (21.4)
Length of hospital stay, days, *Me (Q1–Q3)*	13 (10–16)	13 (10–16)	13 (10–16)	17 (13–21)	16(14–23)	18(13–21)

In patients with a history of COVID-19, the NLR index only correlated with SOFA score and outcome. In the entire patient cohort, an increase in the SIRI index correlated with an increase in SOFA score. This pattern did not persist when patients with/without a history of COVID-19 were analyzed separately. The SII score correlated with the Charlson Index and RAPID scores in the entire patient cohort and in those without previous COVID-19, and with SOFA scores in COVID-19 survivors. An increase in SII correlated with poor prognosis in the entire cohort and in COVID-19 survivors.

We then analyzed the neutrophil, lymphocyte, and monocyte counts in patients in the PE and PEF groups on the 1st, 3rd, 5th, 7th and last day of hospitalization (Fig. 4).

Neutrophils. As shown in Figure 4, there was a significant difference in neutrophil counts between patients with and without fistula on the 5th, 7th, and last days of hospitalization.

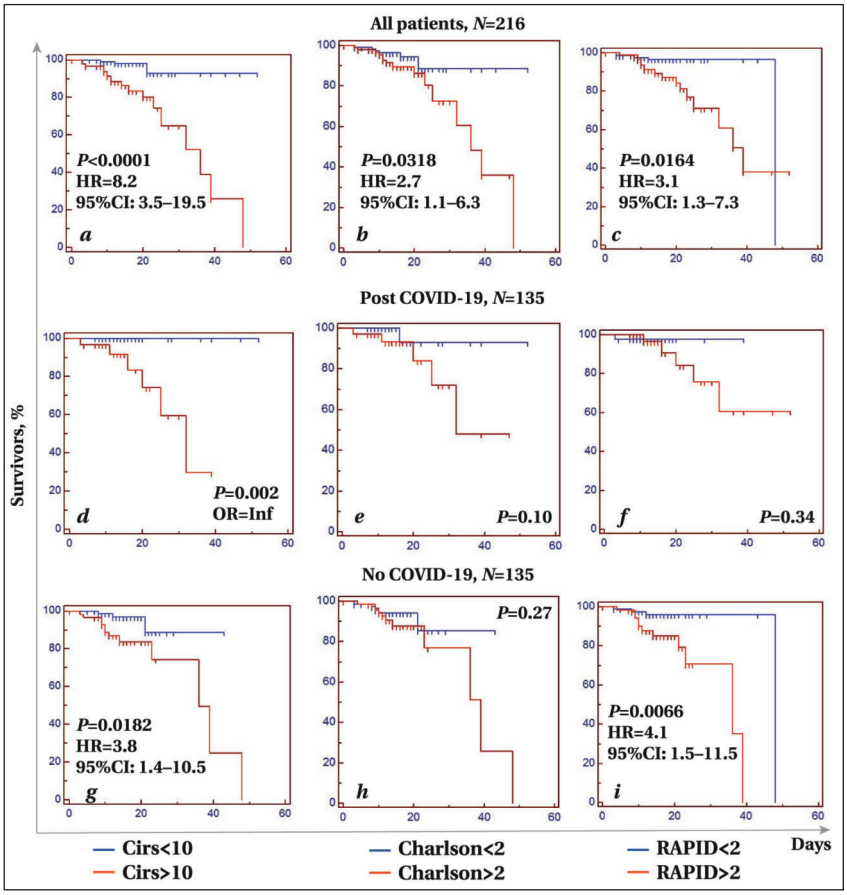

Fig. 3. Prognostic significance of CIRS and RAPID scores, Charlson score for outcome in pleural empyema regardless of fistula formation (Log-rank test).

In both PEF and PE groups, when comparing the circulating neutrophil counts on day 1 of hospitalization between the subgroups of patients with vs without a history of COVID-19, no differences were found (Table 5).

The prognostic value of circulating neutrophils counts for the outcome of SDLP was also analyzed, including those in relation to history of COVID-19 (Fig. 5).

As shown in Figure 5, an elevated neutrophil count was associated with an unfavorable outcome of SDLP in the entire patient cohort (Fig. 5, *a*). However, on separate analysis of patients with/with-

Table 3. Results of linear regression analysis of demographics. scores and cellular parameters.

Parameter	Age	Sex	SOFA	CIRS	Charlson index	RAPID	Lymphocyte count	Neutrophil count	Monocyte count
Age	—	0.13	0.23	0.63	0.77	0.67	–0.12	0.04	–0.18
		0.09	**0.0098**	0.063	<0.0001	**<0.0001**	0.57	0.09	0.35
Sex	0.13	—	–0.02	0.11	0.11	–0.03	0.013	–0.04	–0.07
	0.09		0.67	**0.02**	**0.02**	**0.037**	0.9	0.59	0.34
SOFA	0.23	–0.02	—	0.45	0.40	0.36	–0.26	0.23	–0.20
	0.0098	0.67		**0.02**	0.051	0.16	0.20	**0.0015**	**0.016**
CIRS	0.63	–0.03	0.45	—	0.77	0.58	–0.17	0.09	–0.17
	0.063	**0.015**	**0.02**		**<0.0001**	0.82	0.75	0.9	0.56
Charlson index	0.77	0.11	0.40	0.77	—	0.66	–0.18	0.015	–0.24
	<0.0001	**0.02**	0.051	**<0.0001**		**<0.0001**	0.64	0.58	**0.0498**
RAPID	0.67	–0.03	0.36	0.58	0.66	—	–0.20	0.02	–0.24
	<0.0001	**0.037**	0.16	0.82	**<0.0001**		0.30	0.64	0.07
Neutrophil count	0.04	–0.04	0.23	0.09	0.015	0.02	–0.08	—	0.46
	0.09	0.59	**0.0015**	0.9	0.58	0.64	0.11		**<0.0001**
Monocyte count	–0.18	–0.07	–0.20	–0.17	–0.24	–0.24	0.22	0.46	—
	0.35	0.34	**0.016**	0.56	**0.0498**	0.07	**0.0058**	**<0.0001**	
Outcome	0.16	0.11	0.48	0.38	0.29	0.30	–0.25	0.26	–0.15
	0.08	0.19	**0.0001**	**0.0198**	0.89	0.17	0.17	**0.0005**	**0.0232**

Note. In each column, the upper value corresponds to r values, the lower value corresponds to *P*-values, *N*=216.

Table 4. Results of linear regression analysis of demographics, scores, and cellular parameters in relation to COVID-19 history.

Parameter	Patients														
	Total, *N*=216						History of COVID-19, *N*=81						No history of COVID-19, *N*=135		
	SIRI	SII	NLR	Lym	Nf	Mon	SIRI	SII	NLR	Lym	Nf	Mon	SIRI	SII	NLR
Age	0.03	0.06	0.18	–0.13	0.02	0.02	0.09	0.007	0.07	–0.10	0.05	–0.27	0.0007	0.09	0.22
	0.96	0.53	0.47	0.45	0.98	0.58	0.23	0.18	0.96	0.16	0.36	**0.004**	0.39	**0.019**	**0.02**
Sex	–0.01	0.03	–0.001	–0.05	–0.1	0.07	–0.01	–0.05	–0.02	0.05	–0.004	–0.07	–0.01	0.05	0.005
	0.49	0.29	0.88	0.089	0.31	0.48	0.38	0.58	0.71	0.30	0.94	0.24	0.62	0.35	0.42
SOFA	0.17	0.32	0.47	–0.28	0.37	–0.13	0.17	0.18	0.47	–0.25	0.16	–0.22	0.16	0.38	0.5
	0.0016	0.12	**<0.0001**	0.72	0.47	0.86	0.27	**0.01**	**0.002**	0.54	0.75	0.58	0.23	0.77	**<0.0001**
CIRS	0.06	0.14	0.24	–0.23	0.09	–0.01	0.14	0.07	0.17	–0.13	0.09	–0.25	0.02	0.17	0.27
	0.08	0.26	**0.0002**	0.23	0.70	0.42	0.24	0.13	0.98	0.51	0.28	0.09	0.58	0.31	**0.03**
Charlson	0.03	0.09	0.22	–0.12	0.03	–0.02	0.07	–0.03	0.08	–0.22	0.005	–0.33	0.008	0.12	0.27
index	0.09	**0.03**	**<0.0001**	0.58	0.93	0.54	0.26	0.06	0.73	0.57	0.88	**0.003**	0.31	**0.03**	**0.02**
RAPID	0.11	0.18	0.34	–0.09	0.06	0.05	0.05	0.15	0.12	–0.27	0.07	–0.29	0.11	0.24	0.42
	0.10	**0.01**	**<0.0001**	0.92	0.61	0.55	0.13	0.13	0.60	0.39	0.52	**0.02**	0.39	**0.007**	**<0.0001**
Outcome	0.26	0.18	0.43	–0.29	0.34	–0.14	0.23	0.19	0.46	–0.23	0.22	–0.16	0.16	0.28	0.42
	0.11	**0.012**	**<0.0001**	0.65	0.49	0.12	0.42	**0.002**	**0.023**	0.83	0.12	0.43	0.34	0.14	**0.001**

Note. In each column, the upper value corresponds to the values of *R*, the lower value corresponds to the values of *P*.

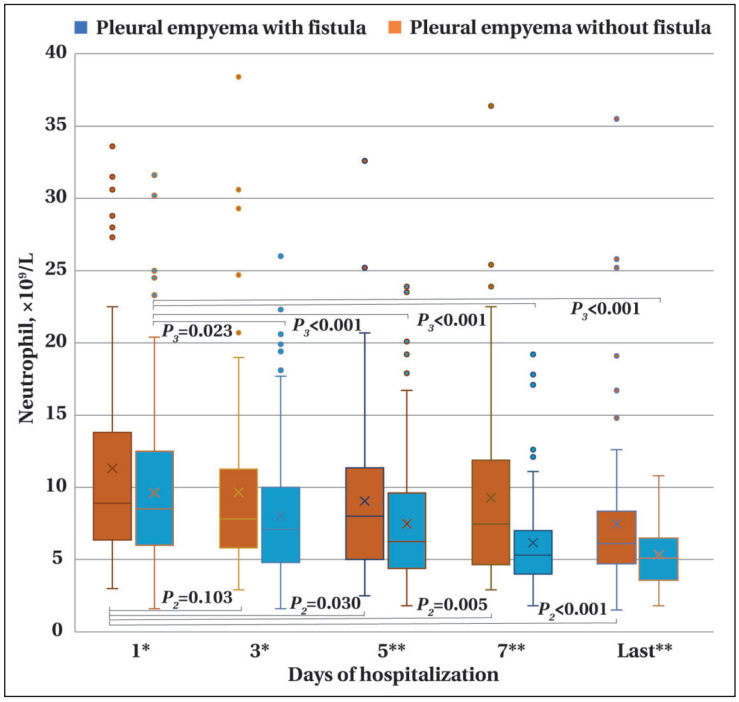

Fig. 4. Neutrophil count in patients with pleural empyema with and without fistula.

Note. ** P_1<0.05; *P_1>0.05. P_1 — significance of differences between neutrophil counts in patients with PE with fistula (blue bars) and without fistula (orange bars) on different days of hospitalization. P_2 — significance of differences between neutrophil counts on different days of hospitalization separately in the PEF group. P_3 — significance of differences between neutrophil counts on different days of hospitalization separately in the PE group.

out COVID-19, we found that an elevated neutrophil count predicted a poor outcome only in patients without a history of COVID-19 (Fig. 5, *c*). For patients with a history of COVID-19, the association was not significant (Fig. 5, *b*).

Lymphocytes. As shown in Table 6, the groups of patients with and without fistula did not differ in lymphocyte counts on days 1, 3, 5, and on the last day of hospitalization. On day 7, patients with pleural empyema with fistula had lower lymphocyte counts than patients with pleural empyema without fistula. Patients with/without a history of COVID-19 in the PEF group had similar lymphocyte counts on days 1, 5, 7, and the last day of hospitalization. However, on day 3 of hospitalization, the lymphocyte count was higher in patients with a history of COVID-19. When comparing the lymphocyte counts in patients in the PE group with/without a history of COVID-19 on the 1st, 3rd, 5th, 7th, and last day of hospitalization, no significant differences were found (Table 6).

We also analyzed the potential contribution of circulating lymphocyte count to the outcome of SDLP (Fig. 5). An increased lymphocyte count was associated with a better prognosis both for the entire patient cohort (Fig. 5, *d*) and for patients with/without a history of COVID-19 (Fig. 5, *e*, *f*). Thus, an increased lymphocyte count during hospitalization may be relevant for a favorable outcome of SDLP, independent of a history of COVID-19.

Monocytes. Monocyte counts in patients with and without fistulas on the 1st, 3rd, 5th, 7th, and last day of hospitalization did not differ (Table 7). There were no differences in monocyte counts between patients with pleural empyema with/without a history of COVID-19.

The associations of neutrophil, lymphocyte, monocyte counts, NLR, SII and SIRI scores with the outcome of SDLP were analyzed using the log-rank test.

The association of patient survival and neutrophil (Fig. 6, *a*), lymphocyte (Fig. 6, *b*) and monocyte (Fig. 6, *c*) counts was found on day 1 of hospitalization.

Table 5. Neutrophil counts in patients with pleural empyema with and without fistula.

Group	History of COVID-19	Values by study days									
		1		3		5		7		Last	
PEF	+	8.9	8.3	7.8	7.6	8.0	7.8	7.0	6.8	6.1	6.1
		(6.3–13.6)	(5.8–11.2)	(5.8–11.2)	(5.7–11.2)	(5–11.2)	(5.2–9.9)	(4.5–11.5)	(5.0–9.8)	(4.7–8.2)	(4.3–7.9)
		N=89	*N*=32	*N*=89	*N*=32	*N*=85	*N*=31	*N*=85	*N*=31	*N*=85	*N*=31
	–		9.7		8		8.4		7.3		6.2
			(6.5–15.9)		(5.8–11.5)		(4.9–11.7)		(4.4–12.5)		(4.7–9.1)
			N=57		*N*=57		*N*=54		*N*=54		*N*=54
PE	+	8.5	8.0	7.2	7.0	6.2	6.6	5.3	5.6	5.1	5.1
		(6–12.4)	(5.5–12)	(4.9–10)	(4.3–9.7)	(4.4–9.6)	(4.2–10.3)	(4.0–7.0)	(3.9–8.0)	(3.6–6.5)	(3.5–6.2)
		N=127	*N*=49	*N*=127	*N*=49	*N*=126	*N*=48	*N*=126	*N*=48	*N*=126	*N*=48
	–		8.6		7.3		6.1		5.2		4.9
			(6.2–12.7)		(5.0–10.1)		(4.5–8.7)		(4.0–7.0)		(3.6–6.6)
			N=78		*N*=78		*N*=78		*N*=78		*N*=78
		P_1=0.179		P_1=0.084		P_1=0.024		P_1≤0.001		P_1=0.001	
		P_2=0.101		P_2=0.489		P_2=0.396		P_2=0.446		P_2=0.565	
		P_3=0.390		P_3=0.482		P_3=0.998		P_3=0.725		P_3=0.947	

Note. For Tables 5–7: reported are *Me (IQR)* and n values for each of the groups (PEF, PE) and subgroups (PEF and a history of COVID-19, PEF without a history of COVID-19; PE and a history of COVID-19; PE without a history of COVID-19) by study day (1st to last). Significance of differences when comparing groups and subgroups: P_1 — PEF vs. PE; P_2 — PEF+ vs. PE+; P_3 — PEF⁻ vs. PE⁻.

Table 6. Lymphocyte counts in patients with pleural empyema with and without fistula.

Group	History of COVID-19	Values by study days									
		1		3		5		7		Last	
PEF	+	1.7	1.9	1.6	1.8	1.5	1.7	1.4	1.5	1.4	1.6
		(1–2.3)	(1.2–2.5)	(1–2)	(1.4–2.2)	(1–1.9)	(1.3–2)	(1–1.9)	(1.1–1.9)	(1–2.1)	(0.9–2.1)
	–		1.6		1.5		1.3		1.2		1.4
			(1–2.3)		(0.9–2)		(1–1.8)		(0.9–1.8)		(1.1–2)
PE	+	1.9	2.1	1.6	1.5	1.6	1.5	1.6	1.5	1.7	1.5
		(1.3–2.3)	(1.1–2.6)	(1.2–2)	(1.2–2.3)	(1.2–2)	(1.1–2.1)	(1.2–2)	(1–2)	(1.2–2.3)	(1.2–2)
	–		1.8		1.7		1.6		1.6		1.8
			(1.3–2.3)		(1.1–2)		(1.2–2)		(1.2–2)		(1.2–2.3)
		P_1=0.292		P_1=0.726		P_1=0.254		P_1=0.010		P_1=0.061	
		P_2=0.479		P_2=0.046		P_2=0.106		P_2=0.126		P_2=0.578	
		P_3=0.561		P_3=0.661		P_3=0.594		P_3=0.598		P_3=0.181	

Increased neutrophil count and decreased lymphocyte and monocyte counts were associated with a poor outcome of SDLP in the entire patient cohort. However, the presence of a prior COVID-19 hospitalization reduced the significance of this association for neutrophil and monocyte counts (Fig. 6, *d*, *f*), but not for lymphocyte counts. The prognostic value of lymphocyte count remained consistent for patients with and without a history of COVID-19 (Fig. 6, *b*, *e*, *h*). In addition, patient survival was significantly associated with lymphocyte count (Fig. 6, *e*, *h*).

In patients without COVID-19, we also found that survival was dependent on the blood monocyte count (Fig. 6, *i*) on day 1 of hospitalization. Thus, a decreased lymphocyte count on day 1 of hospitalization was an adverse prognostic factor regardless of the history of COVID-19, and a decreased monocyte count indicated an unfavorable prognosis only in patients without a history of COVID-19.

Analysis of the relative values of cellular markers of the immune system in all patients of the cohort revealed an association of survival with the values of SII (Fig. 7, *a*), SIRI (Fig. 7, *b*) and NLR (Fig. 7, *c*) on the first day of hospitalization, namely increased values of SII, SIRI and NLR were associated with an unfavorable outcome of SDLP.

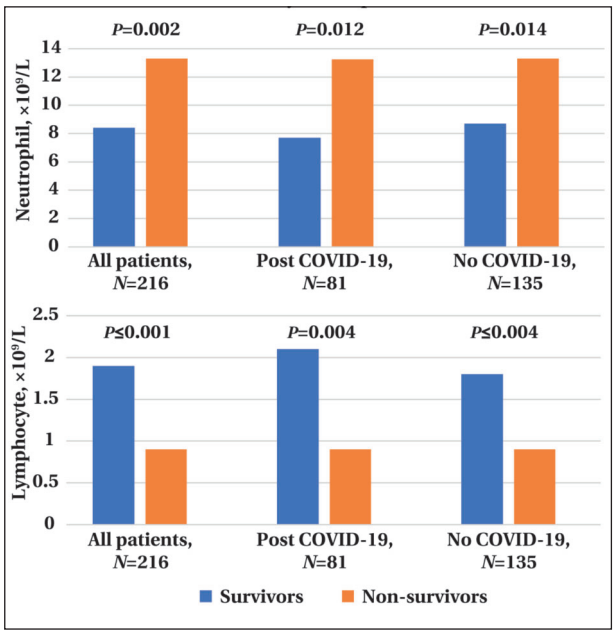

Fig. 5. Outcome of SDLP in relation to neutrophil and lymphocyte count on the 1st day of hospitalization (Mann–Whitney test).

Fig. 7. Prognostic significance of immune cell counts on day 1 of hospitalization.

Group	History of COVID-19	Values by study days									
		1		3		5		7		Last	
PEF	+	0.8	0.7	0.6	0.6	0.5	0.6	0.5	0.5	0.6	0.6
		(0.6–1.2)	(0.6–1.1)	(0.4–0.9)	(0.5–0.8)	(0.4–0.8)	(0.3–0.7)	(0.4–0.7)	(0.4–0.7)	(0.4–0.7)	(0.4–0.8)
	–		0.9		0.6		0.5		0.5		0.6
			(0.5–1.3)		(0.4–1)		(0.4–0.8)		(0.4–0.7)		(0.3–0.7)
PE	+	0.9	0.9	0.7	0.7	0.6	0.6	0.6	0.6	0.5	0.5
		(0.6–1.2)	(0.6–1.1)	(0.5–0.9)	(0.5–0.8)	(0.4–0.8)	(0.4–0.8)	(0.4–0.7)	(0.4–0.8)	(0.4–0.7)	(0.4–0.7)
	–		0.9		0.7		0.6		0.6		0.5
			(0.6–1.2)		(0.5–1)		(0.4–0.7)		(0.5–0.7)		(0.5–0.7)
		P_1=0.480		P_1=0.318		P_1=0.497		P_1=0.624		P_1=0.604	
		P_2=0.541		P_2=0.891		P_2=0.906		P_2=0.916		P_2=0.390	
		P_3=0.634		P_3=0.603		P_3=0.691		P_3=0.794		P_3=0.427	

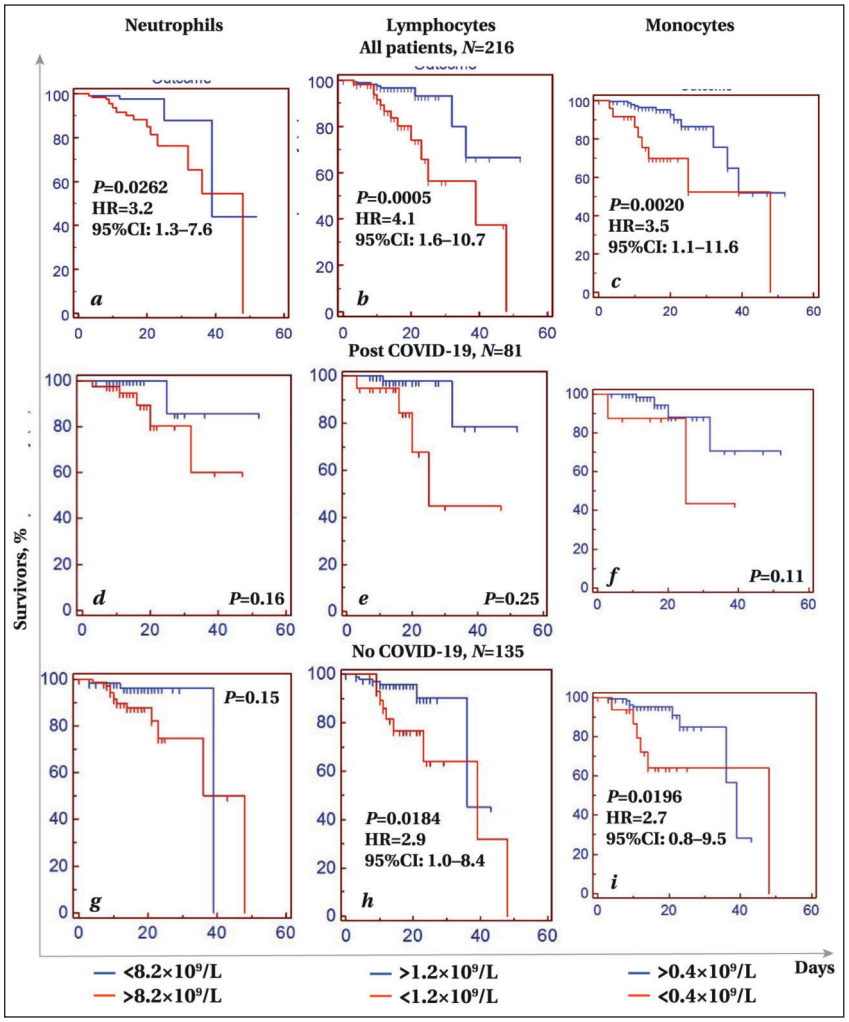

Fig. 6. Prognostic significance of immune cell counts on day 1 of hospitalization.

In patients with a history of COVID-19, there was a significant difference in survival in relation to SII (Fig. 7, *d*), SIRI (Fig. 7, *e*), and NLR (Fig. 7, *f*) on day 1 of hospitalization. No such difference was found in patients without a history of COVID-19 (Fig. 7 *g*, *h*, *i*).

Thus, SIRI, SII, and NLR values were the most informative in predicting the outcome of SDLP in patients with a history of COVID-19 (HR value greater than 7).

The results of the survival analysis based on cell marker levels on day 1 of hospitalization are summarized in Table 8.

Discussion

On the first day of hospitalization, a decrease in lymphocyte count below 1.2×10⁹/L allows predicting an unfavorable outcome of pleural empyema; however, the prognosis does not depend on the patient's history of COVID-19. Another potential marker, monocyte count, could predict the outcome of pleural empyema only in the subset of patients without a history of COVID-19. Increased SIRI (>4), SII (>2500) and NLR (>6), which characterize the severity of the systemic inflammatory response, were associated with the risk of adverse outcomes in patients with SDLP and a history of COVID-19.

The observed significant association of mortality with an increase in NLR on the first day of hospitalization reflects the high prognostic value of both neutrophil and lymphocyte levels (which serve as the basis for calculating NLR values) (Fig. 5, 6). The prognostic value of these markers is understandable given the pathogenetic significance of both cell populations as key components of adaptive defense mechanisms against infection. In response to bacterial infection, neutrophils migrate intensively from the bone marrow and become activated, releasing large amounts of oxygen and nitrogen radicals (ONRs), proteolytic enzymes, and cytokines. Neutrophils undergo netosis, the formation of «neutrophil traps,» which are complexes of positively charged nuclear proteins

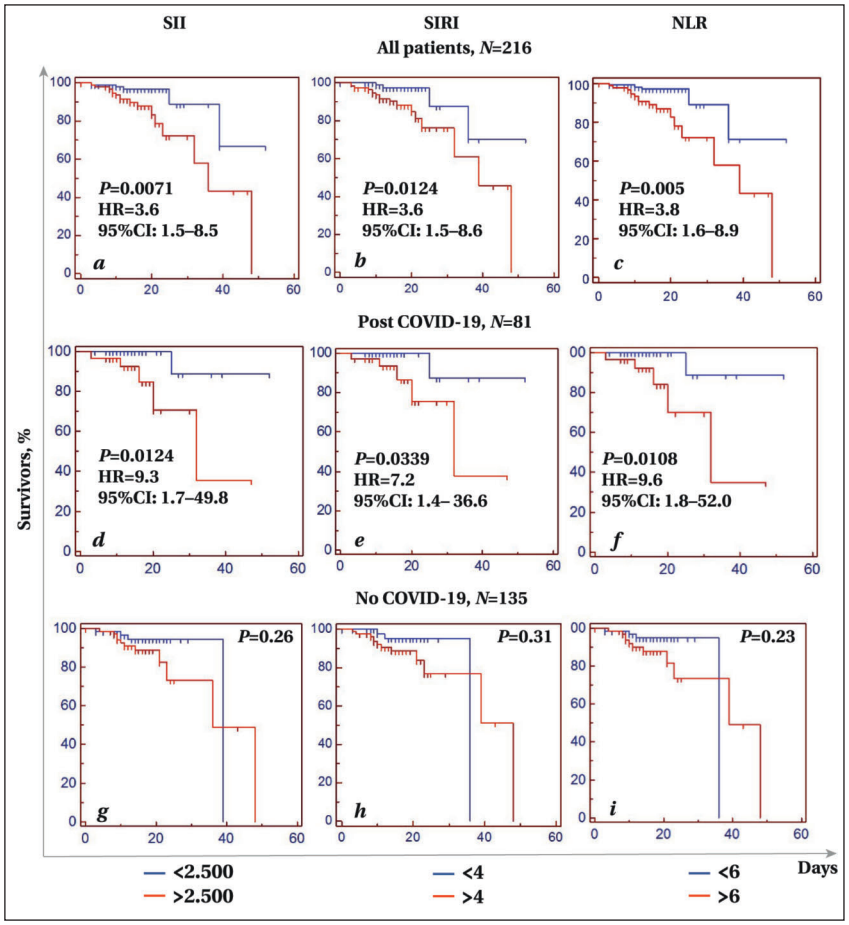

Fig. 7. Values of SII, SIRI, and NLR and the outcome of SDLP.

(HMGB1 and histones) and large fragments of nuclear and mitochondrial DNA. Located on the surface of neutrophils, neutrophil traps bind bacteria, but together with ONRs and the proinflammatory microenvironment, they can cause damage to the vascular endothelium [42]. The latter manifests as degradation of the cell surface glycocalyx and an increase in endothelial permeability due to impaired interactions between endothelial cells. The collapse of endothelial barrier structures consistently leads to increased microvascular permeability, vascular hypotension, edema, decreased tissue perfusion, and the development of life-threatening organ failure typical of septic infectious complications [43]. The associated amplification of procoagulant mechanisms associated with degradation of anticoagulant systems on the surface of endothelial cells and increased expression of tissue factor (TF) may contribute to the severity of the disease [44]. These processes, occurring with underlying progressive endothelial dysfunction, increase the likelihood of thrombotic complications and worsen the prognosis of SDLP.

On the other hand, the decreased lymphocyte count in the blood of patients with SDLP, which is associated with a poor prognosis, indicates a decrease in the immune responses carried out by cells of the adaptive immune system — T and B cells. This increases the risk of an unfavorable outcome due to an increased likelihood of severe infections as a result of decreased immune competence. Lymphopenia and high NLR are recognized as adverse prognostic markers for the progression of pneumonia, including COVID-19-associated pneumonia [45–47]. In contrast, low NLR levels, corresponding to decreased neutrophil counts and increased lymphocyte counts, have been associated with better prognosis in pneumonia [48].

A recent study found that elevated levels of NLR, SII, and SIRI, which are relative biomarkers of systemic inflammatory response, were predictive of COVID-19 outcomes [49]. Patients with SII values above 1835 had a lower oxygenation index and more severe lung changes on CT than those with SII values below 1835.

Neutrophils, the most abundant and diverse circulating granulocytic leukocytes, play an important role in the innate immune system. They serve as the «first line» of immune defense against bacterial and fungal infections, destroying microorganisms by phagocytosis, producing antibacterial peptides

Table 8. Survival analysis in relation to cell marker levels on day 1 of hospitalization using Cox regression.

	All patients			History of COVID-19			No history of COVID-19		
	***P*-value**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**
Lymphocytes	**0.0012**	2.1	1.3–3.2	**0.0026**	0.1	0.01–1.0	**0.0285**	0.4	0.2–1.0
Neutrophils	**0.0199**	1.8	1.0–3.1	**0.0033**	1.2	1.1–1.4	**0.0298**	1.1	1.0–1.2
Monocytes	**0.0071**	2.0	1.2–3.1	0.35			0.14		
SIRI	**0.0091**	1.9	1.1–3.3	0.06			0.057		
SII	**0.0061**	1.9	1.5–3.1	0.08			**0.0096**	1.0	1.0–1.1
NLR	**0.0044**	1.9	**1.2–3.2**	**0.0016**	1.2	1.0–1.3	**0.0009**	1.0	1.0–1.1

and ONRs, and forming NETs. However, their role in viral infections is unclear. Neutrophils in SARS-CoV-infected mice do not appear to be required for virus clearance from lung cells or host survival [50].

Cells with a neutrophil-like phenotype may also have significant immunosuppressive activity [51]. Their increase in circulation is associated with the severity of pneumonia [52]. There are data on the involvement of immunoregulatory cell populations in COVID-19. Here, they perform two opposing functions — they suppress virus-specific T-cell immune response and reduce excessive inflammatory response [53]. However, the prognostic value of detecting this cell population in pneumonia has not yet been established.

The lymphopenia observed in COVID-19 is probably related to the ability of the virus to infect T cells via viral S protein involving angiotensin converting enzyme receptor 2 (ACE2) and possibly CD147 [54]. In COVID-19 disease, a decrease in CD3+, CD4+, and CD8+ T lymphocytes and an increase in regulatory T cells are often observed.

The prognostic significance of a decrease in lymphocyte count on the first day of hospitalization was established in patients without a history of COVID-19 (Fig. 7, *b*, *e*, *h*). This indicates that even a small decrease in lymphocyte count (cut-off point of less than 1.0×10^9/L, not even reaching the lymphopenia limit, i. e., less than 10^9/mL) is of key importance for the course of SDLP. It can be assumed that disturbances of the adaptive T-cell immunity system in COVID-19, possibly caused by «immune exhaustion» after the period of their previous activation, may be of a prolonged nature, being part of the whole complex of various consequences of this disease. It is possible that such patients require immunomodulatory drugs capable of increasing the functional activity of CD4+ and CD8+ T lymphocytes or B cells. However, clinical evidence supporting the use of immunomodulators in the treatment of SDLP is currently lacking, as no large-scale clinical trials of treatment with immunomodulators such as Lycopid©, Immunophan©, Polyoxidonium©, and immunoglobulin-containing preparations have been reported in the literature.

In several diseases, a decrease in CD4+ and CD8+ T lymphocytes is often associated with disease severity and leads to an increase in NLR values. This ratio is considered to be a more sensitive biomarker of clinically significant immune system disorders than neutrophil and lymphocyte counts taken separately [49, 55].

The relative markers NLR, SII, and SIRI seem to reflect systemic inflammation and a wide range of immune responses carried out by innate and adaptive immune cells in an integrated manner. In prolonged or recurrent infections, a sustained inflammatory response can exhaust the immune system, thereby reducing systemic immunity. The reason for the rapid decline in peripheral blood lymphocyte counts in SDLP may be inadequate recovery from COVID-19 or increased susceptibility to immune cell death by apoptosis [56] or pyroptosis characteristic of lung disease [57].

Elevated NLR and CRP levels may predict adverse outcomes in COVID-19 patients [58, 59]. Considering that SARS-CoV-2 can directly infect endothelial cells, an increase in the NLR in COVID-19 may indicate a risk of endothelial dysfunction as a result of the joint damaging effect of the virus and neutrophils on the endothelium, followed by progressive endothelial damage, induction of a proinflammatory cascade with activation of complement factors C3 and C5, increased endothelial permeability, and production of chemokines that increase chemotactic migration of inflammatory cells. It is possible that coronavirus infection preceding SDLP permanently disrupts some components of the innate and adaptive immune systems, predisposing to a greater migratory ability of neutrophils and leading to an increase in the relative indices of innate immunity NLR, SII, SIRI, a decrease in the peripheral blood lymphocyte count, and the ability of the adaptive immune system to resist the increased bacterial load in SDLP [58].

Interestingly, the relative SIRI index, which depends on the increase in neutrophils and monocytes on the one hand and the decrease in lymphocytes on the other, had the potential to predict early death in patients with SDLP and a history of COVID-19 (Fig. 8). This may be due to the marked immunosuppression in these patients, as morphologically granulocytic myeloid-derived suppressor cells (G-MDSC) can belong to the neutrophilic granulocytes and monocytic myeloid-derived suppressor cells (M-MDSC) to the monocytic population [51]. Both subpopulations of immunosuppressive cells are generated during infection, and an association with mortality in septic complications in ICU patients has been found for both G-MDSC [60] and M-MDSC [61].

The ongoing search for pathogenetically significant biomarkers that can aid in the early detection of life-threatening and emergency conditions remains a priority. Researchers hope to find prognostic biomarkers that can stratify patients into risk groups for adverse outcomes of SDLP and allow timely selection of optimal personalized treatment methods. The present study provides simple clinical and laboratory relative cellular biomarkers of SDLP outcomes that are associated with the pathogenesis of lung disease and reflect the levels of immune system cells and may serve as candidate markers for further validation in other studies.

The limitation of our study was its lack of external validity, as it was conducted in a single center.

This highlights the importance of confirming the results in other clinical settings.

Conclusion

In patients with suppurative diseases of lungs and pleura, prior COVID-19 may influence the prognostic value of absolute and relative cellular markers of the immune system. Lymphocyte count on day 1 of hospitalization is a biomarker independent of demographic and clinical variables that can predict the outcome of pleural empyema in patients regardless of prior COVID-19; namely, a decrease in lymphocyte count below 1.2×10^9/mL is associated with mortality.

In patients with pleural empyema and no history of COVID-19, the monocyte count is prognostically significant. Increased levels of the relative cell biomarkers SIRI, SII and NLR on the first day of hospitalization are associated with mortality in patients with COVID-19.

An increase in CIRS comorbidity score above 10 is associated with an unfavorable outcome of SDLP, independent of COVID-19 history.

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Relationship Between Sepsis Phenotypes and Treatment Characteristics of Patients with Viral and Bacterial Pneumonia

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Summary

New subgroups of patients with severe community-acquired pneumonia (SCAP) are hardly predicted by the use of clinical covariates; clusterization may significantly improve diagnostic approaches and facilitate the adaptation of specific treatment modalities to patient's individual characteristics.

The aim of the study. To identify linking the sepsis phenotype in patients with SCAP and preferable treatment option to forecasting the outcome and improve treatment results.

Materials and methods. Case histories of 664 intensive care unit (ICU) patients with sepsis (2016–2023) from I. I. Mechnikov Northwestern State Medical University were analyzed. The study included 568 (85.5%) patients with viral SCAP (SCAPv group) and 96 (14.5%) patients with bacterial SCAP (SCAPb group). Sepsis phenotypes were identified using algorithm proposed by Seymour C.W. et al. In SCAP cases associated with COVID-19 infection (*n*=293, 51.6%) patients received genetically engineered biological therapy (GEBT). The study compared two cohorts of patients: those who received GEBT and did not receive GEBT. Data were statistically processed using the Statistica 10.0 and SPSS software packages.

Results. Analysis revealed 4 sepsis phenotypes: α- (*N*=323, 48.6%); β- (*N*=128, 19.3%); γ- (*N*=87, 13.1%); δ- (*N*=126, 19%). The majority of SCAPv group patients — 295 (51.9%) — had α-phenotype of sepsis, while δ-phenotype prevailed in the SCAPb group — 53 (55.2%). The proportion of patients receiving GEBT and exhibiting α-sepsis phenotype dominated over other sepsis phenotypes: 61.8% of patientspossesed α-phenotype, whereas β-, γ- and δ-phenotypes were determined in 16% , 12.6%, and 9.6% of GEBT patients, respectivelty ($P<0.05$). The best effect of using monoclonal antibodies to interleukin-6 receptors as a GEBT was obtained in patients with the α-phenotype sepsis and COVID-19-associated SCAP: 87.5% favorable outcomes, $P=0.0419$. Rate of bacterial sepsis was significantly lower in patients with α- and δ-phenotypes of sepsis receiving GEBT vs those who did not receive this therapy: 12.71% vs 23.2% of patients with α-phenotype, $P=0.0131$; 25.0% vs 70.41% of patients with δ-phenotype, $P=0.0254$, respectively.

Conclusion. Differences in sepsis phenotype between patients with viral or bacterial SCAP may stratify patients for different therapeutic management and more accurately predict potential complications and unfavorable outcome.

Keywords: sepsis phenotypes; severe community-acquired pneumonia; genetically engineered biological therapy; response; outcome

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

Introduction

Community-acquired pneumonia (CAP) is one of the most common acute infectious diseases [1,2] accounting for a significant proportion of respiratory deaths [3]. The main causes of death in patients with severe community-acquired pneumonia (SCAP) are refractory hypoxemia, septic shock (SS) and organ dysfunction [4]. The host response to sepsis can be variable [5], which may partly explain the clinical heterogeneity that makes early diagnosis and treatment difficult [6]. Since the 2010s, numerous studies have been initiated worldwide to systematize sepsis and septic shock [7–11]. Current research is primarily focused on improving the accuracy of sepsis diagnosis using omics technologies [12], including the development of point-of-care testing systems [13]. Another critical aspect of clinical research is the collection of baseline phenotypes and patient trajectories using multivariate analysis techniques such as principal component analysis [14], factor analysis, and probabilistic [15] or consensus clustering [16]. Deep reinforcement learning has also emerged as an important area of study for assessing the continuum of organ dysfunction in sepsis [17]. The common trend among these initiatives is to assess patient trajectories, which includes investigating the prevalence of each phenotype and its impact on clinical outcomes such as long-term survival, resistance to vasopressor support, and duration of organ support [17–22]. The understanding of sepsis is complex and is aided by a variety of pattern recognition techniques used to identify sepsis subclasses. It should be noted that each newly derived subclass must be evaluated in the following

steps: 1) biological plausibility, 2) ability to predict treatment response, and 3) consistency and reproducibility across data sets [23]. The most reproducible study of sepsis phenotypes to date was that of Seymour et al. «Sepsis ENdotyping in Emergency CAre (SENECA)», which was conducted in multiple cohorts of patients from 12 centers over a 5-year period. The phenotypes were retrospectively replicated in cohorts with different types of sepsis and used in three randomized controlled trials (RCTs): ACCESS, PROWESS, and ProCESS. All three RCTs were multicenter and included extensive clinical data on sepsis biomarkers. The SENECA derivation cohort used matched k-means clustering models and showed that a 4-class model was most effective in distinguishing the α, β, γ, and δ phenotypes. The authors found that the identified sepsis phenotypes were associated with patient response to treatment as well as short-and long-term outcomes [24]. A study analyzed 42,735 patient data from the Multiparameter Intelligent Monitoring in Intensive Care-IV and eICU Collaborative Research Database to evaluate 79 Surviving Sepsis Campaign recommendations for four phenotypes (α, β, γ, and δ) in patients with sepsis and identify the most effective intensive care practices [25]. Bruse et al. studied 52,274 patients with sepsis and COVID-19 as well as three cohorts of patients with sepsis without COVID-19 (non-COVID-19 viral pneumonia sepsis, bacterial pneumonia sepsis, and bacterial sepsis of non-pulmonary origin) and found that dexamethasone was most effective in patients with the δ phenotype. Thus, the identification of phenotypes in SCAP will help to tailor therapeutic techniques [27] to the unique characteristics of patients [28].

The aim of our study was to identify sepsis phenotypes in patients with severe community-acquired pneumonia to improve treatment efficacy and prognosis.

Materials and Methods

During the study, we retrospectively reviewed 664 case histories of patients with SCAP admitted to the intensive care unit of I. I. Mechnikov Northwestern State Medical University between 2016 and 2023. There were 568 (85.54%) patients with viral SCAP and 96 (14.45%) with bacterial SCAP.

Sepsis and/or septic shock were confirmed according to the Sepsis-3 definition (https://jamanetwork.com/journals/jama/fullarticle/2492881). Retrospectively, SCAP phenotypes were differentiated using the algorithm proposed by Seymour et al. [24]. Patients were treated according to the provisional guidelines [29] and the guidelines of the Russian Federation of Anesthesiologists and Intensive Care Physicians [30].

The patients were assessed using Elixhauser Comorbidity Index (a measure of the overall severity of comorbidities that directly predicts length of hospital stay, hospital costs, and mortality), ATS/IDSA (American Thoracic Society/Infectious Diseases Society of America) minor criteria and the following scales: SOFA, APACHE IV (Acute Physiology And Chronic Health Evaluation IV), mNUTRIC (Modified Nutrition Risk in Critically Ill Score), NEWS2 (National Early Warning Score, British standardized assessment of patient severity based on 7 clinical parameters), A-DROP (Age, Dehydration, Respiratory failure, Orientation disturbance (confusion), and low blood Pressure, which is a modified version of the CURB-65 scale), SMART-COP (Systolic blood pressure, Multilobar infiltrate, Albumin, Respiratory rate, Tachycardia, Confusion, low Oxygen, low PH, which is an Australian model to identify patients requiring respiratory support and catecholamine infusion based on 8 clinical features), SAPS II (new Simplified Acute Physiology Score II, designed to assess severity and predict mortality in ICU patients), and GCS (Glasgow Coma Scale).

Data were analyzed using Statistica 10.0, SPSS, and Stat Research software packages at the Statistical Research Center in St. Petersburg, Russia. The Kolmogorov-Smirnov test was used to determine whether the distribution of variables was normal. Quantitative parameters with a normal distribution were expressed as arithmetic mean and standard deviation *($M\pm\sigma$)*. In the case of non-normal distribution, quantitative variables were reported as median *(Me)* and lower and upper quartiles *(Q1–Q3)*. The Mann–Whitney *U*-test was used to compare two independent groups. The Kruskal–Wallis test was used to test quantitative parameters for equality of medians across multiple samples. Qualitative parameters were compared between independent groups using Pearson's χ^2 and Fisher's exact test. The relationship between quantitative parameters was assessed using Spearman's rank correlation test. $P<0.05$ indicated significant differences between values.

Results and Discussion

Sepsis phenotypes were determined using 25 clinical and laboratory characteristics from 29 typical cluster variables in the SENECA data, as proposed by Seymour et al. (2019). The α-phenotype of sepsis had the lowest mortality rate and was the most common (48.6%) in the cohort, supporting the findings of Seymour et al. [24]. Fig. 1 shows the clustering of phenotypes based on 25 clinical and laboratory parameters.

The mean SOFA scores did not differ significantly between the viral and bacterial SCAP groups and was 5.34±2.73 points for all patients, whereas in the original study by Seymour et al. the SOFA score was lower at 3.9 points [24].

Among all patients, 4 sepsis phenotypes were identified: α (*N*=323, 48.6%); β (*N*=128, 19.3%); γ (*N*=87, 13.1%); δ (*N*=126, 19%). The α-phenotype

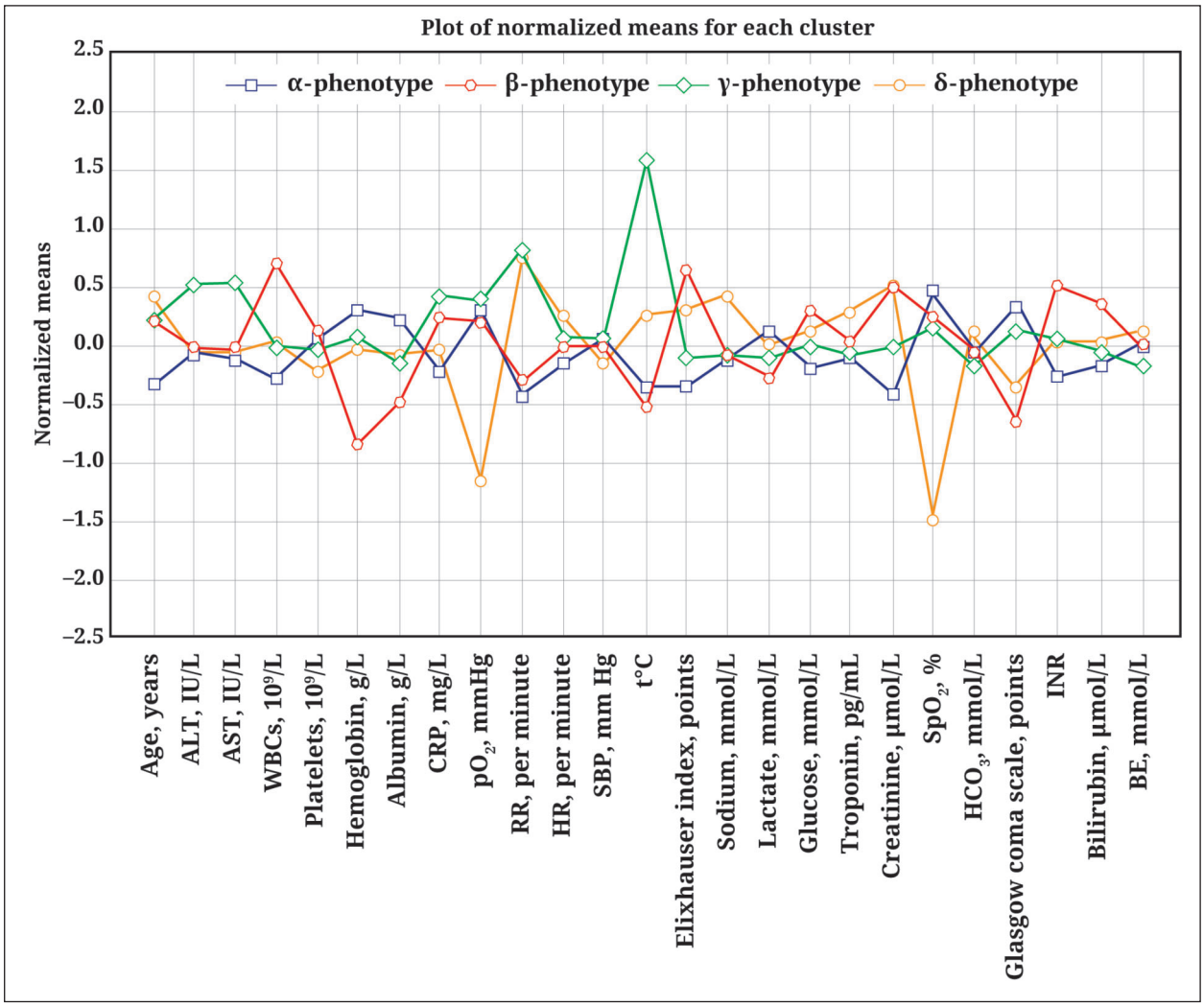

Fig. 1. Clusters of sepsis phenotypes based on 25 clinical and laboratory parameters.
Note. ALT — alanine aminotransferase; AST — aspartate aminotransferase; WBCs — white blood cells; CRP — C-reactive protein; pO_2 — partial pressure of oxygen; RR — respiratory rate; HR — heart rate; SBP — systolic blood pressure; t°C — temperature; SpO_2 — pulse oximetry; HCO_3 — bicarbonate; BE — base excess (or deficit); INR — international normalized ratio.

(*N*=295, 51.9%) was predominant in the vSCAP group, whereas the δ-phenotype (*N*=53, 55.2%) was most common in the bSCAP group.

The proportion of the β-phenotype of sepsis (19.3% of the entire patient cohort) was consistent with the study by Kalimouttou et al. [25], in which patients with the β-phenotype were highly prevalent and had the highest mortality rate (*N*=2022; OR 0.69; 95% CI: 0.50–0.94; *P*=0.01). In contrast, in a study by Bruse et al., in which the percentage of sepsis β-phenotype in the cohorts ranged from 1 to 4%, the α-phenotype was associated with the most favorable outcome, whereas the δ-phenotype was linked to the highest mortality [26].

As shown in Table 1, patients with sepsis α-phenotype were significantly younger in the vSCAP group than in the bSCAP group: 62.2±13.8 years vs. 71.3±13.4 years (*P*=0.001). Females with sepsis δ-phenotype were more frequent in bSCAP compared to vSCAP: 36 (67.9%) vs 34 (46.5%), *P*=0.0173.

Severe comorbidities with a mean Elixhauser Comorbidity Index score ⩾12 points were detected in the δ-phenotype in the vSCAP group and in the β-phenotype in the bSCAP group. Semicoma (SCH ⩽ 11 points) on admission was more common in sepsis β-phenotype in the bSCAP group. The highest score (⩾4 points) on the ATS/IDSA small criteria [31] was seen in sepsis β- and γ-phenotype in the bSCAP group.

Severe elevation of ferritin (1071 (518–1728) µg/L) was recorded in the β-phenotype of sepsis in the vSCAP group. The highest levels of procalcitonin (1.6 (0.5–2.5) ng/mL) and D-dimer (3.2 (2.0–4.4) µg/mL) were found in the γ-phenotype patients of the bSCAP group, and the highest levels of fibrinogen (6.2 (5.0–7.8) g/L) were observed in the γ-phenotype patients of the vSCAP group.

When comparing the APACHE IV scale scores [32] between the vSCAP and bSCAP groups, the highest score in the ß-phenotype of sepsis was recorded in the vSCAP group (126 (116-136) vs 112 (84.5–117.5), *P*=0.0484), and in δ-phenotype, in bSCAP group (123 (95–159) vs 87 (49–125), *P*=0.0014), in contrast to the study of Bruse et al. [26], where patients with β-phenotype sepsis had a mean APACHE IV score of 78 (62–98).

In the bSCAP group, the β-phenotype of sepsis was associated with longer ICU (19 (8.5–35.5) days) and hospital (19 (16.5–47) days) stays, and the longest duration of mechanical ventilation (23.5±27.0 days).

Most patients with β-phenotype sepsis required vasopressor support (6 [85.7%]) and reserve group antibiotics (7 [100.0%]). PaO_2/FiO_2 < 250 mmHg was documented in the majority of patients with α-, β-, γ-, and δ-phenotypes of sepsis in the vSCAP group, whereas only 62.5% of patients in the bSCAP group had similar values.

The lowest need for vasopressor support (norepinephrine at a dose above 0.5 μg/kg/min) was found in patients with the α-phenotype of sepsis in both vSCAP and bSCAP: 18 (6.1%) and 7 (25.0%) patients, respectively, *P*=0.0003. In patients with β-phenotype sepsis, high-dose vasopressor support was used more frequently in the bSCAP group than in the vSCAP group (85.7% vs. 43.8 %, *P*=0.0305).

Reserve group antibiotic use was ⩾85.5% in the α, β, and γ sepsis phenotypes in the bSCAP group. Significant differences in the frequency of reserve group antibiotic use were observed only between patients with α-phenotype sepsis in viral and bacterial SCAP (54 (18.3%) vs. 24 (85.7%) patients, *P*<0.0001). The ß-phenotype in the bSCAP group was associated with sepsis in all patients and invasive candidiasis in 4 (57.1%) patients.

The need for aggressive nutritional therapy with a high mNUTRIC score [33] was higher in patients with bSCAP than in those with vSCAP: α-phenotype 5 (4–6) vs. 3 (3–5) points, *P*=0.0002; β-phenotype 7 (7–8) vs 5 (4–6) points, *P*=0.0055; γ-phenotype 6.5 (4.8–8) vs 5 (3–6) points, *P*=0.0494; δ-phenotype 6 (5–7) vs 5 (4–6) points, *P*<0.0001. A high incidence (mean ⩾56.7%) of pulmonary embolism was observed with the β-phenotype in both SCAP groups. For all sepsis phenotypes, the duration of ICU stay was longer in the bSCAP group than in the vSCAP group (Table 1).

The highest number of poor outcomes was observed in β-phenotype of viral and bacterial sepsis, which was consistent with the study by Kalimouttou et al. [25]. In pairwise comparison, hospital mortality was higher in patients with α- and δ-phenotypes in the bSCAP group compared to those in the vSCAP group: with α-phenotype, 11 (39.2%) vs. 45 (15.2%), *P*=0.0013; with δ-phenotype, 33 (45.2%) vs. 34 (64.1%), *P*=0.0354.

Differences by sepsis phenotype between patients with viral and bacterial SCAP are shown in Table 1.

Clustering into 4 phenotypes of sepsis in COVID-19 SCAP patients in samples receiving (*N*=293) and not receiving (*N*=275) biologic therapy showed differences in severity of illness, length of hospital and ICU stay, complication rates, and mortality (Table 2).

The most commonly identified sepsis phenotype among COVID-19 SCAP patients receiving biologic therapy (BT) was the α-phenotype (*N*=181, 61.8%). They had a higher BMI of 30.6 (26.7–34.7) kg/m² compared to 26.9 (24.2–30.8) kg/m² (*P*<0.0001). A P/F index of 250 mmHg was found in 80.7% of the subjects who received BT and in 46.5% of those who did not receive BT (*P*<0.0001). We discovered that patients who underwent BT exhibited a significantly higher frequency of cancer and COPD compared to those who did not: 5 cases (2.8%) versus 11 cases (7.8%) for cancer (*P*=0.0405), and 5 cases (2.8%) versus 11 cases (7.8%) for COPD (*P*=0.0405), respectively. Patients who did not receive BT had higher D-dimer levels of 0.97 (0.4–2.3) μg/mL vs. 0.4 (0.2–1) μg/mL (P=0.0002), while fibrinogen levels were 6.4 (5.3–7.5) g/L vs. 5.5 (4.2–6.6) g/L (*P*=0.0005).

Patients who did not receive BT had a higher incidence of acute cardiovascular events and bacterial sepsis: 12 (9.7%) vs. 0 (0.0%) (*P*<0.0001) and 33 (23.2%) vs. 23 (12.7%) (*P*=0.0131).

Patients who received BT had a longer time from disease onset to ICU admission and a longer inpatient hospital stay: 10 (8–12) vs. 8 (6–14) days (*P*=0.0090) and 19 (15–27) vs. 18 (12–24) days (*P*=0.0203), respectively.

A NEWS2 [34] score > 8 on admission to the ICU was significantly higher in patients with α-phenotype sepsis who received BT: 172 (95.0%) vs 110 (78.0%) (*P*<0.0001), as well as the A-DROP scale *P*=0.0376) in contrast to the SMART-COP scale score [36] ⩾5 points, 11 (6.1%) vs. 21 (14.9%), (*P*=0.0087). The SAPS II scale score [37] was also statistically significantly higher in patients with α-phenotype sepsis who received BT, 28 (24–35) vs 26 (21–31.8) (*P*=0.0155).

Patients with β-phenotype sepsis represented only 16% (*N*=47) of the sample of patients receiving BT in COVID-19 SCAP and 26.9% (*N*=74) of the sample of patients not receiving BT.

Patients with β-phenotype sepsis who received BT were found to be hospitalized later than those who did not receive BT, at 12 (8–15) and 9 (4–14) days after onset, respectively.

Patients with β-phenotype sepsis were older than those with other sepsis phenotypes and their comorbidities were more severe. All patients with

Table 1. Comparison of sepsis phenotypes in viral and bacterial SCAP.

Parameter	Viral SCAP, *N*=568		Bacterial SCAP, *N*=96						*P*-value			
Phenotype	**α**	**β**	**γ**	**δ**	**α**	**β**	**γ**	**δ**	**P(α)**	**P(β)**	**P(γ)**	**P(δ)**
Total, *N* (%)	295 (51.9%)	121 (21.3%)	79 (13.9%)	73 (12.9%)	28 (29.2%)	7 (7.3%)	8 (8.3%)	53 (55.2%)				
Age, years *(M±σ)*	62.2±13.8	73.8±12.7	70.6±12.1	72.3±11.4	71.3±13.4	73.4±14.6	70.6±16.9	68.9±14.0	0.0010	0.8257	0.7023	0.2038
Sex, *N* (%)												
Women	135 (45.8)	63 (52.0)	33 (41.7)	34 (46.5)	13 (46.4)	6 (85.7)	2 (25.0)	36 (67.9)	0.9461	0.0825	0.3566	0.0173
Assessment scales, *Me (Q1–Q3)*												
SOFA (points)	4 (3–6)	6 (5–8)	6 (4–8)	6 (4–8)	2 (1–4)	2 (1–3.5)	5.5 (4.5– 6.3)	4 (3–7)	<0.0001	0.0004	0.7541	0.0030
EI (points)	4 (0–5)	8 (4–13)	5 (3–10)	12 (5–18)	4 (0–8)	13 (5–16.5)	8 (4–9)	10 (4–18)	0.8734	0.4876	0.4908	0.2854
GCS (points)	15 (15–15)	15 (13–15)	15 (14–15)	14 (13–15)	15 (15–15)	11 (8–15)	14.5 (13.8–15)	14 (8–15)	0.5674	0.1377	0.1641	0.1033
ATS/IDSA: minor criteria (points)	2 (2–2.5)	4 (3–5)	4 (2–4)	3 (2–4)	1 (0–2)	3 (3–4)	3.5 (2.8–4.5)	2 (1–3)	<0.0001	0.1099	0.7777	0.0173
mNUTRIC (points)	3 (3–5)	5 (4–6)	5 (3–6)	5 (4–6)	5 (4–6)	7 (7-8)	6.5 (4.8–8)	6 (5–7)	0.0002	0.0055	0.0494	<0.0001
APACHE IV (points)	52 (47–74)	126 (116–136)	95 (54.5–126)	87 (49–125)	70.5 (56.3–89.8)	112 (84.5–117.5)	110 (91.3–123.3)	123 (95–159)	0.0026	0.0484	0.6171	0.0014
SAPS II (points)	28 (22–34)	36 (31–43)	33 (30–39)	35 (28–40)	33.5 (27.8–38.5)	37 (36–43.5)	37.5 (31.8–37.5)	41 (33–53)	0.0093	0.4438	0.2860	0.0023
Laboratory parameters, *Me (Q1–Q3)*												
Ferritin, μg/L	716 (400–1345)	1071 (518–1728)	971 (455–1649)	987 (454–1767)	680 (680–680)			417.4 (129.3–606.0)	0.9437	1.0000	1.0000	0.0428
Procalcitonin, ng/mL	0.1 (0.1–0.3)	0.4 (0.2–1.2)	0.5 (0.2–3.7)	0.6 (0.2–1.7)	0.6 (0.2–2.3)	1.2 (0.2–3.1)	1.6 (0.5–2.5)	1.4 (0.6–5.3)	0.0001	0.3929	0.4314	0.0058
D-dimer, μg/L	0.5 (0.3–1.4)	1.5 (0.7–3.8)	1.8 (0.6–4.8)	1.8 (0.8–3.5)	1.4 (0.9–1.9)	1.2 (1.2– 1.2)	3.2 (2.0–4.4)	2.9 (0.7–6.4)	0.1751	0.7580	0.5651	0.4594
Fibrinogen, g/L	6.1 (4.7–7.4)	6.0 (4.6–7)	6.2 (5.0–7.8)	6.0 (4.2–7.7)	2.7 (2.1–4.0)	3.8 (3.1–3.8)	4.4 (4.3–5.1)	4.9 (3.1–7.2)	0.0005	0.0317	0.0979	0.2024
Intensive care												
Duration of RS, days, *M±σ*	4.1±4.0	7.7±5.9	5.8±3.9	4.7±4.5	2.8±3.0	23.5±26.9	5.8±4.9	4.8±10.1	0.1647	0.1152	0.9015	0.0150
P/F < 250 mmHg, *N* (%)	208 (70.5)	113 (93.3)	67 (84.8)	61 (83.5)	4 (14.2)	4 (57.1)	5 (62.5)	26 (49.0)	<0.0001	0.0009	0.1114	<0.0001
Vasopressor dose >0.5 μg/kg/min, *N* (%)	18 (6.1)	53 (43.8)	36 (45.5)	13 (17.8)	7 (25.0)	6 (85.7)	6 (75.0)	19 (35.8)	0.0003	0.0305	0.1124	0.0216
Reserve group antibiotics, *N* (%)	54 (18.3)	88 (72.7)	54 (68.3)	45 (61.6)	24 (85.7)	7 (100.0)	7 (87.5)	36 (67.9)	<0.0001	0.1088	0.2596	0.4676
Complications, *N* (%)												
PE	69 (23.5)	68 (56.6)	38 (48.1)	33 (45.2)	1 (3.7)	4 (57.1)	1 (12.5)	7 (13.2)	0.0170	0.9803	0.0537	0.0001
Bacterial sepsis	127 (43.0)	80 (66.1)	51 (64.5)	46 (63.0)	21 (75.0)	7 (100.0)	7 (87.5)	39 (73.5)	0.0012	0.0618	0.1896	0.2112
Invasive candidiasis	76 (25.7)	43 (35.5)	31 (39.2)	25 (34.2)	4 (14.8)	4 (57.1)	2 (28.5)	8 (15.0)	0.2076	0.2489	0.5780	0.0158
Length of stay and outcomes												
Re-transfer, *N* (%)	28 (9.5)	7 (5.8)	11 (13.9)	5 (6.8)	2 (7.1)	0 (0.0)	0 (0.0)	8 (15.1)	0.6824	0.5128	0.2588	0.1331
Days in ICU, *Me (Q1–Q3)*	4 (2–8)	7 (4–12)	7 (4–9.5)	5 (3–8)	10 (6.5–14.5)	19 (8.5–35.5)	8.5 (6.5–11)	6 (2–10)	<0.0001	0.0501	0.4258	0.4038
Days in the hospital, *Me (Q1–Q3)*	18 (14–25)	13 (8–21)	17 (9.5–25)	19 (9–27)	14.5 (10.5–18.3)	19 (16.5–47)	9 (5–10)	11.5 (4.8–20.5)	0.1363	0.1766	0.0586	0.0228
Unfavorable outcome, *N* (%)	45 (15.2)	115 (95.0)	43 (54.4)	33 (45.2)	11 (39.2)	7 (100.0)	6 (75.0)	34 (64.1)	0.0013	0.5462	0.2637	0.0354

Note. RS — respiratory support; PE — pulmonary embolism; EI — Elixhauser Index.

Table 2. Comparison of patients with different sepsis phenotypes in COVID-19 SCAP receiving (*N*=293) and not receiving (*N*=275) biologic therapy.

Parameter	COVID-19 SCAP receiving BT (*N*=293)				COVID-19 SCAP not receiving BT (*N*=275)				*P*-value			
Phenotype	**α**	**β**	**γ**	**δ**	**α**	**β**	**γ**	**δ**	***P*(α)**	***P*(β)**	***P*(γ)**	***P*(δ)**
Total, *N* (%)	181 (61.8)	47 (16.0)	37 (12.6)	28 (9.6)	114 (41.5)	74 (26.9)	42 (15.3)	45 (16.4)				
Patient characteristics												
BMI, kg/m², *Me (Q1–Q3)*	30.6 (26.6–34.6)	28.4 (25.3–31.1)	29.2 (26–33.2)	26.1 (24.1–32.7)	26.9 (24.2–30.8)	25.8 (23.0–29.8)	25.9 (23.5–31.8)	24.5 (23.4–28)	<0.0001	0.0183	0.0274	0.1448
Age, years, *M*±σ	63.8±15.0	74.9±12.7	71.2±12.9	72.9±12.5	61.1±12.8	72.1±12.5	69.9±11.3	71.2±9.6	0.0729	0.2111	0.4060	0.3850
Comorbidities, N (%)												
HTN and CHD	125 (69.0)	43 (91.5)	30 (81.0)	23 (82.1)	94 (66.2)	62 (76.5)	35 (70.0)	79 (80.6)	0.5846	0.0337	0.2398	0.8557
LC	6 (3.3)	4 (8.5)	0 (0.0)	0 (0.0)	4 (2.8)	5 (6.1)	2 (4.0)	13 (13.2)	0.7976	0.6180	0.2184	0.0418
Cancer	5 (2.7)	6 (12.7)	3 (8.1)	7 (25.0)	11 (7.7)	12 (14.8)	8 (16.0)	23 (23.4)	0.0405	0.7479	0.2735	0.8668
COPD	5 (2.7)	4 (8.5)	3 (8.1)	3 (10.7)	11 (7.7)	0 (0.0)	4 (8.0)	8 (8.1)	0.0405	0.0076	0.9854	0.6732
CRF	33 (18.2)	13 (27.6)	10 (27.0)	6 (21.4)	17 (11.9)	9 (11.1)	8 (16.0)	13 (13.2)	0.1226	0.0167	0.2094	0.2871
Assessment scales												
EI (points), *Me (Q1–Q3)*	4 (1–5)	5 (3.5–11)	5 (4–10)	12 (7.3–18.5)	4 (0–5)	10 (4–14)	6 (0–10.8)	12 (5–18)	0.4909	0.0126	0.9723	0.7759
GCS (points), *Me (Q1–Q3)*	15 (15–15)	15 (15–15)	15 (15–15)	15 (13–15)	15 (15–15)	13.5 (12–15)	15 (14–15)	14 (13–15)	0.0293	<0.0001	0.0102	0.7897
NEWS2 (points), *Me (Q1–Q3)*	9 (8–10)	10 (8–11.5)	9 (8–10)	9 (8–10)	8 (8–8)	9.5 (8–11)	9 (8–10)	8 (8–10)	<0.0001	0.8153	0.9755	0.2754
NEWS2 > 8 points, *N* (%)	172 (95.0)	47 (100.0)	36 (97.3)	27 (96.4)	110 (78.0)	69 (85.2)	43 (86.0)	59 (60.2)	<0.0001	0.0056	0.0714	0.0003
ATS/IDSA: minor criteria, (points), *Me (Q1–Q3)*	2 (2–3)	4 (3–4)	3 (2–4)	3 (2–3.3)	2 (1–2)	4 (3.3–5)	4 (2–5)	3 (2–4)	0.0088	0.0762	0.1219	0.8515
A-DROP ≥5 points, *N* (%)	17 (9.4)	9 (19.2)	2 (5.4)	6 (21.4)	5 (3.5)	16 (19.8)	5 (10.0)	9 (9.2)	0.0376	0.9338	0.4360	0.0776
SMART-COP ≥5 points, *N* (%)	11 (6.1)	13 (27.7)	6 (16.2)	8 (28.6)	21 (14.9)	38 (46.9)	15 (30.0)	49 (50.0)	0.0087	0.0320	0.1374	0.0445
SOFA (points), *Me (Q1–Q3)*	4 (3–6)	6 (4–8)	4 (3–6)	6 (4–8)	4 (4–6)	6 (5–9)	6 (5–8)	6 (4–8)	0.5191	0.0755	0.0002	0.8707
mNUTRIC (points), *Me (Q1–Q3)*	3 (3–5)	5 (4–6)	4 (3–5)	5 (3–6)	3 (3–5)	5.5 (4–6.8)	5 (4–6)	5 (4–6)	0.5420	0.1561	0.0010	0.2674
APACHE II (points), *Me (Q1–Q3)*	5 (5–14)	25 (25–26)	18 (5–25)	21 (5–25)	5 (5–14)	25.5 (25–28)	25 (6.75–26)	16 (5–25)	0.9268	0.4106	0.0781	0.4915
APACHE IV (points), *Me (Q1–Q3)*	54 (47–69)	125 (103–133)	88 (48–121)	91.5 (54.2–125)	49.5 (47–74)	127 (120–136)	113 (56.7–129)	76 (49–126)	0.7486	0.3115	0.0389	0.8692
SAPS II (points), *Me (Q1–Q3)*	28 (24–35)	33 (30.5–39)	32 (30–39)	35.5 (26–40.3)	26 (21–31.8)	38 (32–44.8)	34 (31–38.8)	34 (30–40)	0.0155	0.0021	0.5449	0.8736
Clinical and laboratory parameters												
SBP, mmHg (minimal), *Me (Q1–Q3)*	92 (69–100)	65 (51.5–100)	59 (56–100)	96.5 (83.7–100.2)	85 (69–97.7)	59 (45–84.7)	69 (48–93.7)	80 (56–96)	0.1210	0.0343	0.7374	0.0282
P/F < 250 mmHg, *N* (%)	146 (80.6)	47 (100.0)	31 (83.7)	25 (89.3)	66 (46.5)	70 (86.4)	41 (82.0)	62 (63.3)	<0.0001	0.0082	0.8276	0.0086
Lymphocytes (minimal), 10^9/L, *Me (Q1–Q3)*	0.8 (0.5–1)	0.6 (0.3–0.8)	0.7 (0.5–0.9)	0.65 (0.5–0.8)	0.9 (0.6–1.3)	0.6 (0.4–1.1)	0.6 (0.4–1)	0.6 (0.3–0.9)	0.0025	0.1006	0.4389	0.9864
Platelets below 100×10^9/L, *N* (%)	4 (2.2%)	5 (10.6%)	3 (8.1%)	1 (3.6%)	11 (7.8%)	14 (17.3%)	10 (20.0%)	14 (14.3%)	0.0189	0.3080	0.1240	0.1226
Ferritin, µg/L, *Me (Q1–Q3)*	905 (493–1550)	1247 (544–1923)	1171 (547–1888)	1150 (740–1863)	456 (323–975)	984 (456–1590)	711 (440–1202.5)	697.5 (341.25–1438.5)	<0.0001	0.4491	0.0655	0.0624
Procalcitonin, ng/mL, *Me (Q1–Q3)*	0.1 (0.1–0.2)	0.2 (0.1–0.4)	0.3 (0.1–0.6)	0.2 (0.1–0.7)	0.2 (0.1–0.5)	0.7 (0.4–2)	2.4 (0.3–7.2)	1.2 (0.4–4.1)	0.1061	<0.0001	0.0001	0.0005
Albumin, g/L, *Me (Q1–Q3)*	35 (32–38)	32 (29–35)	35 (32–37)	29 (26–32)	34 (30–38)	30 (25.7–32.3)	28 (25–31.8)	26 (22–30)	0.2244	0.0052	<0.0001	0.3495
CRP (maximal), mg/L, *Me (Q1–Q3)*	84 (41.5–147.6)	110 (66–167)	127.2 (63.9–185)	111.7 (75.5–167.6)	54.1 (18.6–128.9)	120 (73.2–169)	157 (119.9–204)	140.7 (81–175.9)	0.0023	0.6671	0.1568	0.4562
D-dimer, µg/mL, *Me (Q1–Q3)*	0.4 (0.2–1)	0.9 (0.5–3.0)	1 (0.4–3.9)	2.8 (1.0–5.0)	1.0 (0.4–2.3)	2.0 (1.1–4)	2.1 (0.9–5.2)	1.4 (0.8–2.9)	0.0002	0.0039	0.1016	0.0908
Fibrinogen, g/L, *Me (Q1–Q3)*	6.4 (5.3–7.5)	6.6 (5.4–7.5)	6.6 (5.8–8.2)	5.3 (4.0–6.9)	5.4 (3.9–7.2)	5.5 (4.2–6.6)	6.1 (4.6–7.1)	6.4 (4.4–8.1)	0.0005	0.0025	0.0661	0.1008
Troponin T, ng/mL, *Me (Q1–Q3)*	13 (8–28.5)	110.5 (34.8–580.2)	48 (23.1– 91)	73.5 (40–327.5)	19.8 (8.5–58)	104.7 (48.9–268.5)	39 (19–149)	71.2 (25–126.7)	0.1252	0.9837	0.9831	0.1859

Continuation Table 2.

Parameter	COVID-19 SCAP receiving BT (*n*=293)				COVID-19 SCAP not receiving BT (*n*=275)				*P*-value			
Phenotypes	**α**	**β**	**γ**	**δ**	**α**	**β**	**γ**	**δ**	***P*(α)**	***P*(β)**	***P*(γ)**	***P*(δ)**
Intensive care												
Duration of RS, days, *M±σ*	4.9±4.4	10.0±5.9	6.5±3.3	6.0±5.5	2.6±2.7	6.1±5.3	5.2±4.3	3.9±3.6	<0.0001	<0.0001	0.9262	0.1912
Dose of vasopressin >0.5 μg/kg/min, *N* (%)	16 (8.8)	23 (48.9)	19 (51.4)	5 (17.9)	4 (3.5)	35 (47.3)	17 (40.5)	10 (22.2)	0.9018	0.8545	0.6214	0.0074
Reserve group antibiotics, *N* (%)	27 (14.9)	33 (70.2)	22 (59.4)	13 (46.4)	51 (35.9)	62 (76.5)	39 (78.0)	68 (69.3)	<0.0001	0.4300	0.0618	0.0253
Complications, *N* (%)												
PE	44 (24.5)	27 (58.7)	13 (35.1)	17 (60.7)	26 (18.4)	45 (55.5)	26 (52.0)	23 (23.5)	0.1871	0.7314	0.1179	0.0002
CVE	0 (0.0)	1 (2.2)	3 (8.1)	0 (0.0)	12 (9.7)	7 (8.9)	4 (8.7)	11 (12.8)	<0.0001	0.1407	0.9237	0.0465
Bacterial sepsis	23 (12.7)	34 (72.3)	26 (70.3)	7 (25.0)	33 (23.2)	65 (80.2)	39 (78.0)	69 (70.4)	0.0131	0.3030	0.4122	<0.0001
Invasive candidiasis	50 (27.6)	24 (51.0)	14 (37.8)	10 (35.7)	30 (21.3)	23 (28.4)	19 (38.8)	23 (23.5)	0.1909	0.0103	0.9295	0.1937
Length of stay, frequency of re-transfers and outcomes												
Days from hospital to ICU admission, *Me (Q1–Q3)*	3 (1–6)	4 (1–6.5)	1 (1–3)	5.5 (1–12)	1 (1–2)	1 (1–4)	2 (1–7.75)	3 (1–10)	<0.0001	0.0513	0.0571	0.9023
Days from disease onset to ICU admission, *Me (Q1–Q3)*	10 (8–12)	12 (8–15)	9 (4–13)	10 (7.5–15.7)	8 (6–14)	9 (4–14)	8.5 (6–16)	9 (7–13)	0.0090	0.0576	0.2258	0.3109
Re-transfer, *N* (%)	15 (8.3)	6 (12.8)	7 (18.9)	2 (7.1)	15 (10.5)	1 (1.2)	4 (8.0)	11 (11.2)	0.4842	0.0057	0.1298	0.5312
Unfavorable outcome, *N* (%)	32 (17.7)	45 (95.7)	19 (51.3)	14 (50.0)	24 (16.9)	77 (95.0)	30 (60.0)	53 (54.0)	0.8545	0.8601	0.4213	0.7027
Days in ICU, *Me (Q1–Q3)*	5 (3–9)	10 (6–14)	9 (6–10)	6 (5–10)	3 (2–5)	6 (3–10)	5.5 (2.25–9)	4 (2–7)	<0.0001	0.0004	0.0289	0.0049
Days in hospital, *Me (Q1–Q3)*	19 (15–27)	17 (12.5–5.5)	16 (10–25)	20 (14.3–24.5)	18 (12–24)	11 (5.3–17)	17.5 (9–24.5)	17 (8–28)	0.0203	<0.0001	0.9529	0.2399

Note. HTN — hypertension; CHD — coronary heart disease; LC — liver cirrhosis; COPD — chronic obstructive pulmonary disease; CRF — chronic respiratory failure; PE — pulmonary embolism; CVE — cardiovascular events; CRP — C-reactive protein; NEWS2 — National Early Warning Score; ATS/IDSA — American Thoracic Society Criteria for Defining Severe Community-acquired Pneumonia; A-DROP — Age, Dehydration, Respiratory failure, Orientation disturbance (confusion), and low blood Pressure; SMART-COP — Systolic blood pressure, Multilobar infiltrate, Albumin, Respiratory Rate, Tachycardia, Confusion, low Oxygen, low PH; RS — respiratory support; SBP — systolic blood pressure; EI — Elixhauser Index; GCS — Glasgow Coma Scale.

β-phenotype sepsis had a P/F index less than 250 mmHg.

When comparing patients with β-phenotype sepsis who received BT to those who did not, the mean Elixhauser Index score was lower in those on BT at 5 (3.5–11) vs. 10 (4–14) (P=0.0126). Their Glasgow Coma Scale score was higher and corresponded to clear consciousness at 15 (15–15) vs 13.5 (12–15) points (P<0.0001). Patients who received BT had higher fibrinogen levels, 6.6 (5.4–7.5) g/L versus 5.5 (4.2–6.6) g/L (P=0.0025). Patients not receiving BT had higher levels of procalcitonin at 0.7 (0.4–2) ng/mL vs. 0.2 (0.1–0.4) ng/mL (P<0.0001) and D-dimer at 2.0 (1.1–4) μg/mL vs. 0.9 (0.5–3.0) μg/mL (P=0.0039). Patients with ß-phenotype receiving BT had a significantly longer duration of respiratory support (10.1±6.0 vs. 6.1±5.3 days, P<0.0001). Invasive candidiasis was diagnosed in 24 (51%) patients receiving BT versus 23 (28.4%) patients not receiving BT (P=0.0103). The rates of bacterial sepsis and pulmonary embolism were similar in both groups.

When we compared the severity of illness in patients with β-phenotype sepsis who received BT and those who did not, we found NEWS2 > 8 in 100.0% vs. 85.2% (P=0.0056) and SAPS II of 33 (30.5–39.0) vs. 38 (32.0–44.8) (P=0.0021), respectively. Patients with the β-phenotype of sepsis who received BT and those who did not had the same rate of adverse outcomes (95.7% vs. 95.0%, P>0.05), but a higher number of adverse outcomes than other sepsis phenotypes.

Patients with the γ sepsis phenotype in both samples had comparable age, Elixhauser Index, ATS/IDSA minor criteria, and SAPS II score. Patients with the γ phenotype who did not receive BT had higher mNUTRIC and APACHE IV scores of 5 (4–6) vs. 4 (3–5) (P=0.001) and 113 (56.7–129) vs. 88 (48–121) (P=0.0389), respectively. Patients who did not receive BT had significantly higher procalcitonin levels, 2.4 (0.3–7.2) ng/mL vs 0.2 (0.1–0.4) ng/mL (P<0.0001), and a shorter mean ICU stay, 5.5 (2.3–9) vs 9 (6–10) days (P=0.0289).

Patients with the δ-phenotype of sepsis in both samples were comparable in age, Glasgow Coma Scale, ATS/IDSA minor criteria, mNUTRIC scale, APACHE IV, and SAPS II. Elixhauser Index and D-dimer levels were higher than in other phenotypes. Pulmonary embolism was the most com-

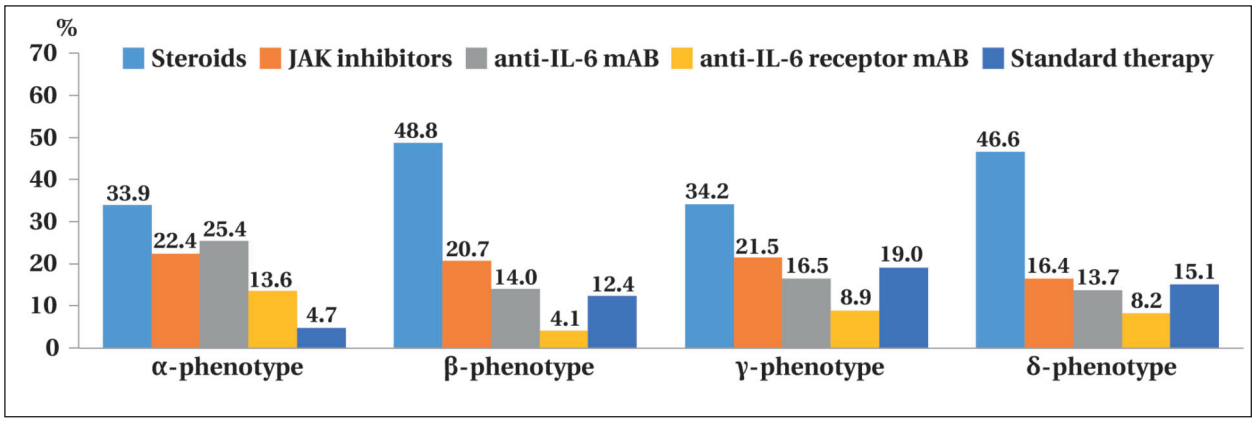

Fig. 2. Treatment of patients with COVID-19 SCAP.
Note. For Figures 2 and 3: mAB — monoclonal antibodies.

mon complication in 17 (60.7%) patients receiving BT and 23 (23.5%) patients not receiving BT (P=0.0002). Bacterial sepsis was reported significantly less frequently in patients who received BT compared to those who did not, 25.0% vs. 70.4%, respectively (P=0.0254).

The treatment of patients with COVID-19 SCAP is summarized in Fig. 2.

Patients with δ-phenotype sepsis had a lower frequency of BT: 13.7% were treated with monoclonal antibodies against interleukin-6 (mAB IL-6) and 8.2% with monoclonal antibodies against interleukin-6 receptor (mAB rIL-6). Steroid therapy (dexamethasone) was used in 46.6% of patients. BT was most frequently used in patients with α-phenotype sepsis, with 25.4% receiving IL6 mAB (olokizumab) and 13.6% receiving rIL6 mAB (tocilizumab, sarilumab).

Comparison of outcomes in α-phenotype sepsis by therapy is shown in Fig. 3.

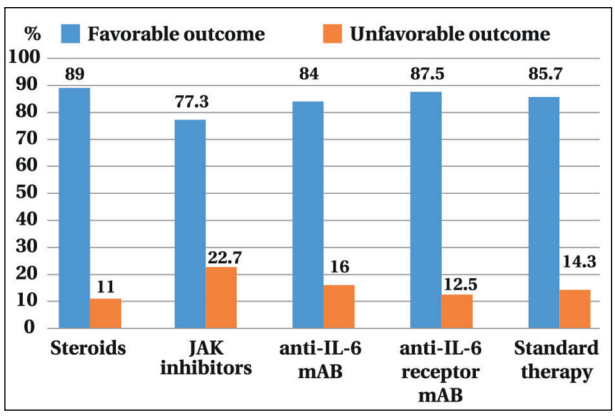

Fig. 3. Comparison of outcomes in α-phenotype sepsis by therapy.

Patients with α-phenotype sepsis who received BT (N=181, 61.8% of sample) had a higher baseline severity of illness than patients who did not receive BT (N=114, 41.5% of sample), as confirmed by severity stratification scoring systems: NEWS2 > 8 points, 172 (95.0%) vs. 110 (78.0%) (P<0.0001); A-DROP ⩾5 points, 17 (9.4%) vs. 5 (3.5%) (P=0.0376); SAPS II, 28 (24–35) vs. 26 (21.0–31.8) points (P=0.0155). Patients receiving BT had a longer ICU stay than those not receiving BT, 5 (3–9) vs. 3 (2–5) days (P<0.0001), with comparable adverse outcomes of 32 (17.7%) vs. 24 (16.9%) (P>0.05).

Bacterial sepsis was significantly less common in patients receiving BT: 12.7% of patients with BT vs. 23.2% without BT (P=0.0131).

Interleukin-6 receptor monoclonal antibody therapy was associated with a favorable outcome in 87.5% of patients with α-phenotype sepsis in the COVID-19 SCAP (P=0.0419).

A favorable outcome was also observed with the use of JAK inhibitors in 11 patients with α-, γ-, δ-phenotypes, moderate COVID-19 severity (CT-2 and NEWS=7 points) and severe comorbidities with an Elixhauser Index score of 4 (1–5).

Conclusion

We retrospectively identified four sepsis phenotypes (α — 48.6%, β — 19.3%, γ — 13.1%, δ — 19.0%) in 664 patients with viral and bacterial SCAP. We identified an association between sepsis phenotypes and SCAP progression, treatment strategies, and outcomes.

We found that the α sepsis phenotype predominated in the vSCAP group (N=295, 51.9%) and the δ-phenotype predominated in the bSCAP group (N=53, 55.2%).

We found that the frequency of BT was higher in the α-phenotype sepsis than in other phenotypes, with 61.8% in the α-phenotype, 16% in the β-phenotype, 12.6% in the γ-phenotype, and 9.6% in the δ-phenotype (P<0.05).

Patients with α- and δ-phenotypes of sepsis who received biological therapy (BT) developed

bacterial sepsis significantly less often than those who did not receive BT: in the α-phenotype 12.71% vs. 23.2% (P=0.0131), in the δ-phenotype 25.0% vs. 70.41% (P=0.0254).

In patients with α-phenotype sepsis and COVID-19 SCAP, interleukin-6 receptor monoclonal antibody therapy was associated with a favorable outcome in 87.5% of cases (P=0.0419).

Our data contribute to the development of a more differentiated approach to patient management and improve the prediction of complications and outcomes in SCAP.

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Diagnosis and Intensive Care in Children's Diabetic Acidosis: an Interdisciplinary Viewpoint

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Summary

Diabetic ketoacidosis (DKA) is the main cause of death and disability in children with type I diabetes mellitus (T1DM). Children's mortality from T1DM reaches 1% in developed countries and 13% in developing countries. The main cause of death in DKA is cerebral edema, clinical manifestations of which develop in 0.5–0.9% of children with DKA, while mortality riches 24%.

Objective. Developing recommendations to prevent life-threatening complications of children with DKA using analysis of literature data and consolidated opinion of experts on the issues of intensive care in children with T1DM.

Materials and methods. We analyzed and discussed studies in diagnosis and treatment of DKA in children with type 1 diabetes and 1200 literature sources since January 1970, published in Russian peer-reviewed scientific journals and international publications presented in the online repository Medline (Pubmed). The search for publications was carried out using the keywords: «children», «DKA», «DM1», «dehydration», «cerebral edema».

Results. We considered issues of epidemiology, pathogenesis, clinical manifestations, diagnosis, intensive care for DKA, as well as clinical and diagnosis, treatment, prevention of cerebral edema issues in children. Limitations of the study were the small number of modern studies with a high level of evidence (randomized controlled trials, meta-analyses) over the past 5 years on DKA in children.

Conclusion. Taking into account the national and international experience, joint recommendations on a consensus format were developed and formulated for the diagnosis of DKA, its leading complications and treatment recommendations for children with T1DM and DKA. Timely and accurate diagnosis of DKA, intensive therapy options based on proven therapeutic efficacy, laboratory and clinical monitoring are warranted to interrupt the DKA pathogenesis, prevent the development of life-threatening conditions, and improve treatment outcomes for children with DKA.

Key words: type 1 diabetes mellitus; diabetic ketoacidosis; children; dehydration; cerebral edema; intensive therapy

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

Introduction

Diabetic ketoacidosis (DKA) is a life-threatening complication of type 1 diabetes mellitus (T1DM), which develops due to absolute insulin insufficiency, manifested by dehydration, hyperglycemia, metabolic acidosis, ketonemia and ketonuria, and can lead to cerebral edema and death if not diagnosed and treated in time [1, 2].

Federal Law 323 «Fundamentals of Health Protection of Citizens of the Russian Federation» defines

clinical guidelines as «documents containing structured information based on scientific evidence on prevention, diagnosis, treatment and rehabilitation, including protocols of patient management (treatment protocols), options for medical intervention and algorithm of actions of a health care provider based on the course of the disease, complications and comorbidities» [3]. In the Russian Federation, clinical guidelines «Type 1 diabetes mellitus in children» have been in effect since 2019, providing brief information on the diagnosis and treatment of DKA, without addressing intensive care. Treatment of children with DKA remains the domain of anesthesiologists and intensive care specialists.

In view of the above, there is an urgent need to summarize the accumulated experience and literature data on DKA in children with a focus on intensive therapy, diagnosis and management of complications.

The goal of the Russian Consensus is to formulate guidelines through a collaborative review of existing literature and expert opinion in the intensive care of pediatric diabetic ketoacidosis (DKA) to prevent serious complications. The goals of this collaboration between endocrinologists and intensive care specialists include:

1) Reviewing and summarizing both international and Russian literature, including clinical guidelines, standards of care, algorithms, and protocols that currently present different and sometimes conflicting methods of management of pediatric DKA, with the goal of finding a unified stance among endocrinologists and intensivists.

2) Summarizing the basic principles of insulin therapy for diabetes, with the goal of mimicking the natural insulin release patterns seen in individuals without diabetes.

3) Establishing a consensus on the importance of glucose as the primary fuel for insulin-dependent tissues and the role of insulin in reducing and halting the production of ketones, which are critical in the management of the metabolic acidosis seen in DKA.

4) Reevaluating current methods of managing dehydration in DKA by gaining a better understanding of the underlying mechanisms of hypovolemia, including the often overlooked aspect of chronic dehydration. This goal includes challenging conventional emergency protocols for managing hypovolemic shock resulting from rapid fluid loss.

5) Creating and implementing a straightforward intensive care protocol for DKA that is both understandable and feasible for healthcare providers at all levels of care, specifically designed for the unique healthcare environment of the Russian Federation.

The adoption of local protocols specifically tailored to the management of pediatric diabetic ketoacidosis (DKA), based on clinically validated diagnostic and treatment strategies, has been shown to significantly minimize instances of noncompliance with national standards and reduce the likelihood of adverse outcomes. The approach to treating children with DKA has recently evolved from broad, aggressive interventions to more tailored treatments. This includes a shift toward prioritizing subcutaneous over intravenous insulin administration at the earliest opportunity, favoring oral rehydration over intravenous fluids, using isotonic (0.9%) saline for fluid replacement, and employing the Holiday-Segar formula for calculating daily fluid needs.

Epidemiology

DKA is a leading cause of death and long-term disability in children diagnosed with type 1 diabetes mellitus (T1DM). The prevalence of DKA in pediatric patients with T1DM varies widely across demographic groups and has been reported to range from 13% to 80% [9–13]. In developed countries with advanced healthcare systems, DKA mortality rates are reported to be as low as 1.0%, while in countries with lower socioeconomic status, this figure rises to between 3% and 13% [14, 15]. Cerebral edema is the most common cause of death in DKA cases, occurring in 0.5–0.9% of pediatric DKA patients and resulting in a mortality rate of 21–24% [16–19].

The frequency of DKA episodes in children with T1DM is lower in more economically developed regions than in less developed regions. The incidence of DKA is also higher in rural areas than in urban areas and their suburbs [20,21]. Factors such as low body mass index, ethnic minority, age between 6 and 15 years, HbA1c $\geqslant$ 8.87%, non-use of short-acting insulin and continuous glucose monitoring systems, presence of nephrotic syndrome, severe hypoglycemia or hypoglycemic coma, autoimmune thyroiditis and COVID-19 have been associated with an increased risk of DKA in children [22, 23]. In addition, children with a history of DKA have been found to have lower IQ scores than their peers with T1DM who have not experienced DKA [24].

Etiology and Pathogenesis

Pathogenesis of diabetic ketoacidosis. DKA occurs predominantly with the onset of T1DM due to insulin deficiency. This condition involves the autoimmune destruction of islet cells in the pancreas, coupled with a surge of insulin-antagonistic hormones (such as glucagon, catecholamines, cortisol, and growth hormone) in the bloodstream. This results in stimulated glycogenolysis and gluconeogenesis in the liver and kidneys, decreased tissue glucose uptake, and progressive elevation of blood glucose levels and hyperosmolarity. Inadequate availability of glucose, which is essential for energy production in cellular mitochondria through oxidative phosphorylation, results in increased lipolysis

and ketogenesis (production of acetone, beta-oxybutyric acid, acetoacetic acid). This sequence of events results in metabolic acidosis and elevated blood ketone levels. When blood glucose levels rise significantly (more than 10 mmol/L above normal), in addition to elevated ketone levels, osmotic diuresis is induced which is associated with loss of electrolytes such as sodium, potassium, phosphorus, and magnesium, as well as water. These losses are exacerbated by vomiting, which is common in severe ketosis. Dehydration from these losses can lead to tissue hypoperfusion, which promotes lactate accumulation and lactate acidosis [25–32,13,19].

Etiology and pathogenesis of cerebral edema in DKA. A serious complication of DKA is cerebral edema, a potentially fatal condition that can occur about 12 hours after the start of intensive DKA treatment, although it sometimes develops before treatment begins. While therapeutic errors in the treatment of DKA can lead to brain edema, it's important to recognize that this condition is not necessarily iatrogenic.

The underlying causes of cerebral edema in DKA are not completely understood. However, several key factors are known to be involved, including increased permeability of the blood-brain barrier, swelling of cerebral astrocytes, and dysfunction of cell membranes. Recent theories have proposed a «two-hit» mechanism involving initial ischemia followed by reperfusion. The «first hit» is ischemia: high blood glucose leads to dehydration and osmotic diuresis, which increases blood osmolarity and results in metabolic acidosis. This condition is compensated by respiratory alkalosis and reduced carbon dioxide levels, resulting in prolonged vasospasm in the brain, which induces cerebral ischemia and impairs the self-regulation of cerebral blood flow. With the administration of fluids and insulin during treatment, these factors are reversed, with a decrease in blood osmolarity and normalization of CO_2 levels after prolonged low levels, which can lead to cerebral hyperemia.

The second «hit» is osmotic and vasogenic cerebral edema. Changes in blood osmolarity and increased cerebral blood flow, along with increased capillary permeability, lead to the development of leak syndrome, which underlies vasogenic edema. Osmotic edema occurs with a rapid decrease in blood osmolality (reduction in glycemia), while the concentration of osmotically active substances in cells (primarily glucose) normalizes more slowly [33].

Brain dysfunction in DKA is also associated with impaired glutamatergic and dopaminergic systems, as evidenced by a significant increase in the concentration of autoantibodies to the glutamatergic NMDAR1 type 1 receptor and the dopaminergic DAR 2 type 2 receptor, especially in children with severely impaired consciousness [35, 36]. Children with cognitive impairment after DKA are characterized by low levels of antioxidant protective enzymes, such as superoxide dismutase and glutathione peroxidase, confirming the role of oxidative stress in brain dysfunction [37].

Factors that increase the risk of cerebral edema in children with DKA include

1. Younger age (less than 5 years).
2. Newly diagnosed diabetes (almost 3 times higher risk).
3. Longer duration of DKA prior to treatment.
4. Prolonged hyperglycemia, high blood urea concentration, severe hypocapnia, metabolic acidosis with low pH, increased blood urea nitrogen [38, 39].

Computed tomography changes (lateral ventricular narrowing) are found in 50–100% of patients on admission to hospital, but only 4–15% of them have mental status disorders [40, 41].

Therapeutic errors that contribute to the development of cerebral edema include

1. Excessive rate and volume of fluid therapy [1].
2. Inappropriate composition of fluid solutions.
3. Inappropriate use of sodium bicarbonate.
4. Rapid lowering of glycemia (>5 mmol/L/hour) [19].

Diagnosis

Signs and symptoms.

Dehydration. Varying degrees of dehydration have been reported in DKA [19]. Severe dehydration is associated with a significant decrease in pH, base depletion, anion gap, increased urea, and diastolic hypertension [42]. The severity of dehydration is assessed using the dehydration assessment scale (Table 1). Assessment of the severity of dehydration, fluid and body weight deficits is approximate.

Recommendation. The severity of dehydration should always be assessed during hospitalization and treatment.

Changes in respiration. In severe cases, tachypnea, deep sighing breathing, respiratory rhythm disturbances, and even Kussmaul breathing may be observed [43, 44].

Gastrointestinal syndrome. It is caused by irritation of the peritoneum by released ketones. Its manifestations include nausea, vomiting, and abdominal pain, which may lead to misdiagnosis of surgical or infectious gastrointestinal conditions, inappropriate patient referral, and delayed medical care [45].

In diabetic patients, vomiting may be associated with esophagitis with fungal mucosal lesions [45].

Recommendation. If pain syndrome persists, diagnostic esophagogastroduodenoscopy should be performed.

Drowsiness and impaired consciousness. The level of consciousness (alertness, activity) is assessed according to the Glasgow Coma Scale (Table 2).

Table 1. Dehydration assessment scale.

Signs	Severity (frequency)		
	Mild (≤5%)	**Moderate (6–9%)**	**Severe (≥10%)**
General appearance	Thirsty, restless, alert	Thirsty, drowsy, postural hypotension	Drowsy, limp, cold, sweaty, cyanotic extremities
Radial pulse	Normal rate and strength	Rapid and weak	Rapid, thready, sometimes impalpable
Respirations	Normal	Deep, may be rapid	Deep and rapid
Anterior fontanelle	Normal	Sunken	Very sunken
Systolic blood pressure	Normal	Normal or low	Low
Skin elasticity	Pinch retracts immediately	Pinch retracts slowly	Pinch retracts very slowly
Eyes	Normal	Sunken	Grossly sunken
Tears	Present	Absent	Absent
Mucous membranes	Moist	Dry	Very dry

Note. After [34, 81].

Recommendation. Level of consciousness should always be assessed in patients with DKA.

Acute kidney injury. The incidence of acute kidney injury (AKI) in children with DKA is 41.5–47%. Moderate to severe and severe AKI are reported in 15.5%, and the incidence decreases with age. AKI is more common in children with DKA with a level of consciousness less than 14 points on the Glasgow Coma Scale and a high blood chloride level [46]. The incidence of severe AKI is as high as 28% [47]. The underlying causes of AKI in DKA are fluid depletion and hyperglycemia leading to renal tubular injury and inflammation. Low pH, serum bicarbonate and corrected sodium, high glycemia and urea nitrogen, and male sex are also risk factors for renal injury [46–50]. In DKA, AKI is associated with hyperchloremia in 90% of patients compared to 56% in children without AKI. Chloride is directly and significantly correlated with length of hospital stay, blood creatinine level, and albumin/creatinine ratio [51]. The median time to development of AKI from the onset of DKA is 13.21±6.78 hours [48]. Dialysis is required in 4% of patients with DKA. AKI in DKA is characterized by favorable outcomes, but the long-term effects of AKI are not fully understood [47]. AKI in pediatric DKA is associated with the development of cerebral edema [52].

DKA is characterized by aminoaciduria. Urinary amino acid levels are highest at the onset of DKA and then decrease. During the first 8 hours of DKA, urinary levels of histidine, threonine, tryptophan, and leucine are highest [53].

Recommendation. In patients with DKA, creatinine and urea, markers of AKI, should be measured.

Laboratory signs. The predominant ketone body in DKA is β-oxybutyric acid (β-oxybutyrate). The proportion of acetoacetate is 15–40%. The laboratory reaction with nitroprusside, which is widely used to detect ketone bodies, detects only acetoacetate, which may underestimate the true levels. Importantly, patients receiving anticonvulsant treatment with valproic acid may have a false-positive nitroprusside test. Ketones appear earlier in the blood than in the urine, making their determination in the blood more meaningful [13].

Recommendation. The laboratory criterion for the development of DKA in a child is hyperglycemia >11 mmol/L with a pH<7.3.

Recommendation. The severity of DKA should be assessed.

Severity of diabetic ketoacidosis is defined according to Table 3. The principles of intensive care do not depend on the severity of DKA.

Laboratory criteria for control of diabetic ketoacidosis

a) pH > 7.3;

b) serum bicarbonate (SB) > 15 mmol/L [45].

Table 2. Assessment of activity in children.

Sign	Response in children		Points
	<1 year old	**≥1 year old**	
Best eye response	To sound	To sound	3
	To pain only	To pain only	2
	No response	No response	1
Best verbal response	«Cooing» or babbling	Spontaneous, conscious	5
	Excited scream	With a delay	4
	Scream in response to pain	Individual words	3
	Moaning in response to pain	Individual sounds	2
	No response	No response	1
Best motor response	Spontaneous or purposeful movements	Obeys commands	6
	Withdrawal on touch	Localizing response	5
	Withdrawal on pain	Withdrawal on pain	4
	Abnormal flexion to pain	Flexion to pain	3
	Abnormal extension to pain	Extension to pain	2
	No response	No response	1

Note. Summarized from [82].

Treatment

Antibacterial and antifungal therapy. Routine antibacterial and antifungal therapy is not used; it is prescribed only when an infection is detected [45].

Principles of intensive therapy. The main components of intensive care in DKA are

1) insulin therapy
2) fluid therapy
3) control of electrolyte disturbances.

Fluid and insulin therapy in DKA has been shown to prevent complications (multiple organ dysfunction with acute kidney injury, rhabdomyolysis, pancreatitis, arrhythmias) and poor outcomes [54].

Insulin therapy. *Recommendation.* Only short-acting insulin or an ultra-short acting human insulin analog should be administered intravenously [43, 44].

Bolus insulin administration is not recommended because of the increased risk of cerebral edema. The mechanism of cerebral edema in this case is a rapid decrease in blood plasma osmotic pressure and worsening of hypokalemia. For ease of use, the calculated dose of insulin (1 unit of insulin per 1 kg of body weight) is diluted with solvent until the final volume of the solution is 20 mL. At this dilution, an infusion rate of 1 mL/h is equivalent to an insulin infusion rate of 0.05 U/kg/h.

Recommendation. The recommended initial insulin infusion rate is 0.05–0.1 U/kg/h.

A lower dose of insulin (0.05 U/kg/h) than the standard dose (0.1 U/kg/h) has also been shown to be effective [55, 56].

When choosing the dose of insulin, it is important to remember that the goal of ketoacidosis treatment is to achieve a consistent reduction in acidosis rather than a decrease in blood glucose. The criterion for adequate insulin therapy (along with appropriate fluid therapy), according to expert consensus, is an increase in BE of at least 5 mmol/L over 6 hours. In the absence of such progress, treatment strategies should be reconsidered.

Recommendation. Insulin should be started at the same time as fluid therapy or at least one hour after fluid therapy is initiated.

Adjustment of the insulin rate (dose) and glucose infusion is based on changes in glycemia during the interval between measurements [43, 44].

The strategy depends on the changes in glycemia and the current fluid therapy and includes the following principles.

1. In patients receiving saline only (first stage of treatment, before glucose infusion):

a) if glycemia does not decrease or increases by more than 5 mmol/L, the rate of insulin administration should be increased by 0.025 U/kg/hour;

b) if glycemia decreases by less than 5 mmol/L, do not change the delivery rate;

c) if glycemia decreases by more than 5 mmol/L, start glucose without changing the insulin delivery rate. Reducing the insulin dose at this stage may halt the resolution of ketoacidosis or even cause its progression.

2. In patients already receiving glucose-containing solutions:

a) if glycemia decreases by less than 5 mmol/L/hour, do not change the insulin delivery rate;

b) if glycemia decreases by more than 5 mmol/L/hour, decrease the insulin delivery rate by 25% or increase the glucose infusion rate. The choice depends on the severity of the ketoacidosis: if it decreases, the insulin dose should be reduced (but not below 0.05 U/kg/h); if it persists, the rate of glucose infusion should be increased;

c) if there is no change in glycemia, increase the insulin infusion rate by 0.025 U/kg/h or decrease the glucose infusion rate. The strategy depends on the severity of the ketoacidosis: if it decreases, the dose of glucose should be decreased; if it persists, the rate of insulin infusion should be increased.

3. Complete withdrawal of intravenous insulin is not recommended until the metabolic acidosis is reversed (minimum dose, 0.025 units/kg/hour).

4. Upon normalization of pH and/or disappearance of urinary ketones, the patient is switched to subcutaneous insulin injections (according to standard regimens), and intravenous insulin is discontinued 30–40 minutes after the first subcutaneous injection.

The rate of glycemic lowering of 5 mmol/L per hour is critical and can lead to fatal cerebral edema. At the same time, no significant differences in neurologic outcomes of DKA and residual neurologic impairment were found when the rate of glycemic reduction was increased to 5.5 mmol/L/h [57]. However, we do not recommend allowing a glycemic lowering rate greater than 5 mmol/L/h.

Recommendation. The optimal rate of glycemic lowering is 1–2 mmol/L per hour, and the acceptable rate is up to 3–5 mmol/L per hour.

Recommendation. During the management of DKA, a safe level of glycemia should be 10–15 mmol/L.

Transcutaneous continuous glucose monitoring in children with DKA is a promising method of glycemic control. However, glycemic values measured

Table 3. Severity of diabetic ketoacidosis.

Degree	Values	
	pH	SB, mmol/L
Mild	<7.3	<15
Moderate	<7.2	<10
Severe	<7.1	<5

Note. After [83].

by this method differ by 11.33–13.40% (average 13.20%) from capillary glucometry values. This may be due to acidosis and decreased blood bicarbonate, which affect the accuracy of monitoring [58].

Fluid therapy. *Recommendation.* Fluid therapy should be continued until pH normalizes (7.35–7.45).

The rate of resolution of ketoacidosis depends on its severity [59]. Rapid fluid administration has been shown to result in more rapid normalization of anion gap, blood sodium, and pCO_2 than slow fluid administration in DKA, which reduces the risk of cerebral edema but is associated with the frequent development of hyperchloremic acidosis. The use of 0.9% sodium chloride compared with 0.45% solution reduces potassium levels more slowly due to a greater increase in chloride levels [60].

Recommendation. The approach to fluid therapy depends on the severity of dehydration and glycemia.

Volume of fluid therapy

Fluid therapy for DKA consists of 2 steps:

1. Primary «fluid resuscitation» (Table 4).
2. Rehydration therapy to replace the remaining deficit.

Table 4. Volume of fluid for primary fluid resuscitation.

Dehydration	
Mild	Not administered
Moderate	10–20 mL/kg in 30–60 minutes, then MFR
Severe	1020 mL/kg in 20 minutes (if there is no effect, repeat up to 2 times), then MFR

Note. Consensus opinion of the authors based on guidelines [45]. MFR — maintenance fluid requirement.

Primary «resuscitation» by fluid infusion:

Performed with isotonic (0.9%) sodium chloride solution only [42, 43, 61].

The use of high (>15 mL/kg) or low (<5 mL/kg) bolus doses not affect the rate of resolution of AKI in children with DKA [62].

For mild dehydration, oral fluids are given if the patient can tolerate the water load. Oral rehydration solutions are preferred [43, 44].

Maintenance fluid requirements can be calculated using Table 5. In overweight children, the calculation is based on ideal body weight for actual height [43, 44].

Table 5. Calculation of the daily maintenance fluid requirement.

Body weight	Required volume	
	Hourly	Daily
≤10 kg	4 mL/kg/h	100 mL/kg/24 hrs
11–20 kg	40 mL + 2 mL/kg/h for each kg from 11 to 20	1000 mL + 5 mL/kg/24 h for each kg from 11 to 20
>20 kg	60 mL + 1 mL/kg/h for each kg >20	1500 mL + 20 mL/kg/24 h for each kg >20

Note. Data summarized after [84].

Excessive fluid volume leads to delayed recovery of renal function in AKI and to the development of hyperchloremia [52].

Fluid therapy. *Recommendation.* For fluid composition, fluid adjustment, and insulin therapy based on changes in glycemia are shown in Figure.The issue of adjusting the composition of fluid therapy should be addressed every 2 hours of therapy after glycemia has been controlled. The use of balanced electrolyte solutions compared with isotonic solutions has been shown in small randomized trials to result in faster reversal of acidosis [63, 64]. There are no statistically significant differences in the length of hospital stay, the incidence of cerebral edema, and the rate of recovery of consciousness when a liberal infusion strategy is used compared with a restrictive strategy [65].

Recommendation. Potassium chloride solution should be added to infusion therapy [43, 44].

Treatment of electrolyte disturbances.

Sodium. The majority of patients with DKA have normal or decreased serum sodium levels.

Hyponatremia (<130 mmol/L) may result from

- hemodilution due to displacement of free fluid from interstitial tissues
- secondary hypoaldosteronism (decreased adrenal cortical function), in cases of extremely late referral to a healthcare provider
- pseudohyponatremia due to elevated blood lipid levels.

In most cases, hyponatremia can be treated without the use of high sodium solutions. As plasma volume is replenished and osmotic diuresis is abolished, aldosterone levels normalize, transmembrane Na^+-K^+ exchange stabilizes, and additional sodium administration is rarely required [45].

Hypernatremia may be caused by compensatory hyperaldosteronism leading to increased sodium reabsorption in response to hypovolemia. Significant hypernatremia is associated with severe cerebral edema and is a poor prognostic indicator [33,39,45].

Fluid therapy has been shown to reduce high sodium and chloride levels. A high infusion rate significantly reduces the initially elevated sodium concentration only 12 hours after the start of therapy, even when 0.45% sodium chloride solution is used. The Glasgow Coma Scale level of consciousness was similar in patients with spontaneous sodium reduction and patients with sodium reduction after fluid therapy. The sodium concentration in the infusion fluid and the ratio of water to sodium losses at the onset of DKA both affect sodium levels. There was no correlation between sodium reduction during fluid therapy for DKA and the severity of mental status disorders [18, 45].

Recommendation. Correct hypernatremia only with 5% glucose solution (an isotonic solution without sodium or hydrochloric acid) until plasma sodium levels return to normal.

Potassium. In ketoacidosis, the following processes occur:

• transmineralization, in which intracellular potassium is replaced by protons
• loss of urinary potassium with polyuria.

As a result, the measurement of plasma potassium does not accurately represent the total depletion of the body's potassium reserves, which generally fall to levels 2–3 times lower than the daily potassium requirement [45].

Potassium chloride solution is added at the rate of 40 mmol K^+ per 1 liter of fluid at the earliest 2 hours after the start of infusion therapy if the documented blood potassium concentration is <5.3 mmol/L. For accurate dosing, 1 mL of KCl 7.5% solution contains 1 mmol K^+, while 1 mL of KCl 4% solution contains approximately 0.5 mmol K^+.

Recommendation. Do not use potassium chloride solution if potassium concentration ≥6.5 mmol/L and anuria (hourly urine output ≤0.5 mL/kg/h).

Recommendation. If initial hypokalemia is less than 3 mmol/L, administer 0.5 mmol/kg potassium chloride solution before starting insulin therapy.

Monitor blood potassium at least every 6 hours. If necessary, administer additional intravenous potassium chloride at a rate not to exceed

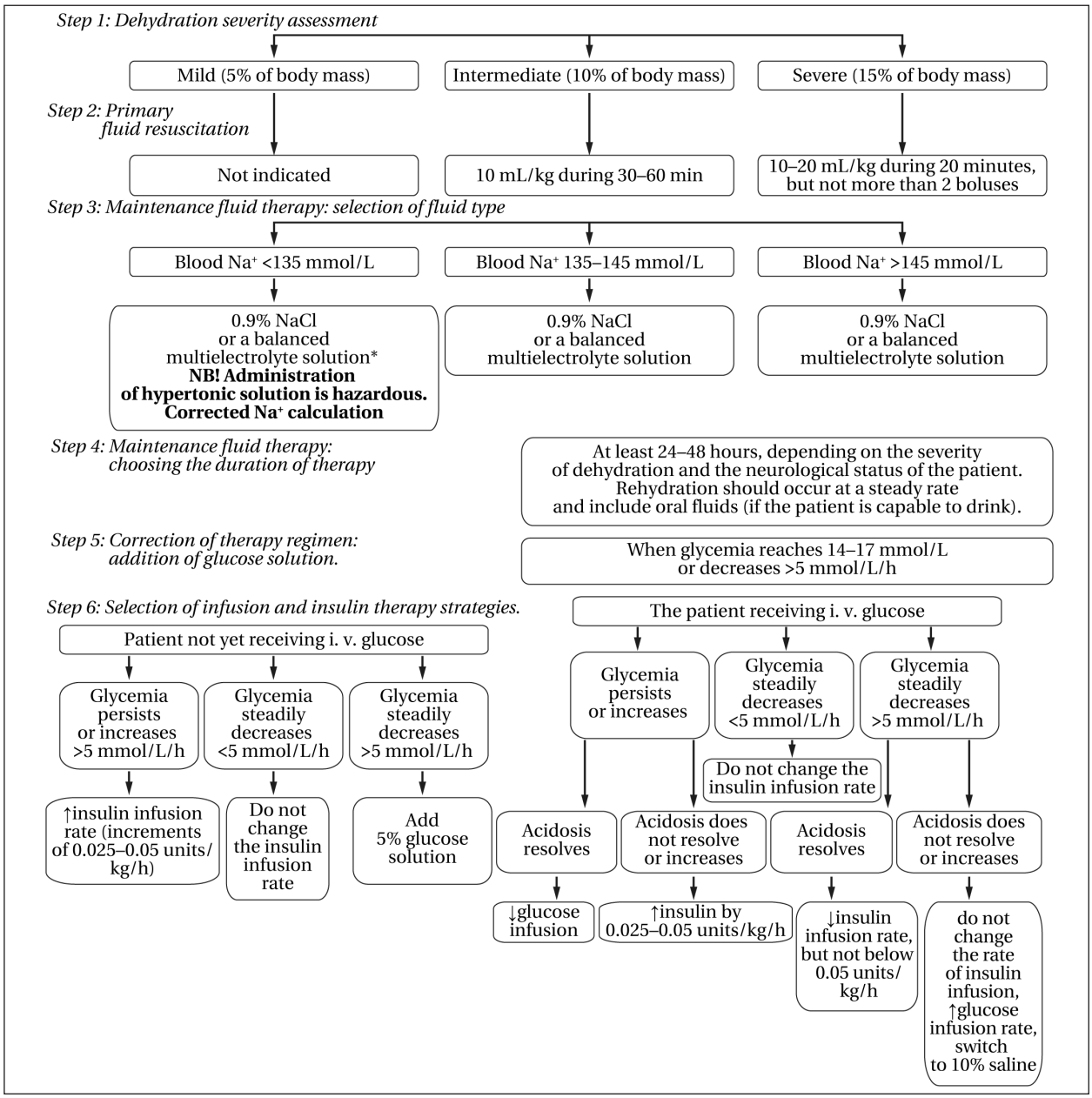

Fig. Fluid therapy algorithm for diabetic ketoacidosis (original illustration).
Note. A 0.45% NaCl solution can be used. For glycemia above 17 mmol/L, starting with 5% dextrose solution has been shown to be effective [28]. Insulin therapy is started concurrently with fluid therapy according to the «4 NOTs» rule: 1) do not give a bolus; 2) do not reduce the rate below 0.05 U/kg/h until the acidosis is stabilized; 3) do not stop the insulin infusion until the ketoacidosis is completely reversed (the minimum insulin infusion rate is 0.025 U/kg/h); 4) do not switch the patient to total subcutaneous insulin until 30 minutes after the first subcutaneous injection.

0.5 mmol/kg per hour, including potassium administered in glucose solution [45].

The use of potassium magnesium aspartate (Panangin®, Asparkam®, KMA®) does not increase blood potassium concentration and cannot correct hypokalemia.

Recommendation. The administration of sodium bicarbonate solutions in DKA is not recommended!

These studies suggest that bicarbonate has no clinical benefit in DKA [66–69]. The use of bicarbonate may result in paradoxical central nervous system acidosis [70–72].

Recommendation. At a minimum, no negative clinical or laboratory changes such as worsening mental status, increasing glycemia, hypocapnia, or decreasing pH should be observed in DKA from the first hour.

If no improvement or negative clinical and laboratory changes are observed, the following causes should be considered:

1) technical, such as proper dilution of insulin solutions, placement of venous catheters, adherence to the prescribed rate of fluid administration by infusion pumps, etc;

2) clinical, such as comorbidities and nutritional or insulin therapy errors that can cause both the onset and slow progression of DKA. The most common of these are

- otolaryngologic conditions (e. g., otitis media, sinusitis), which may present with less obvious manifestations;
- intestinal infection;
- acute surgical abdominal conditions (abdominal pain should not always be interpreted solely as a manifestation of ketoacidosis);
- urinary tract infection requiring urinalysis and examination of the patient's external genitalia for signs of balanoposthitis, vulvovaginitis, bartholinitis;
- soft tissue infections such as perineal inflammation, which may progress to phlegmon or abscess.

Patient routing. *Recommendation*. Children with DKA should be treated in a tertiary T1DM center or under its guidance.

Hospitalized children with DKA and pH=7.07±0.07 without mental status impairment can be safely managed in non-intensive care units [73].

Recommendation. Children with DKA and pH<7.3 with impaired consciousness below 14 on the Glasgow Coma Scale should be treated in an intensive care unit.

Patients should be consulted by intensivists of a regional intensive care consultation center or a remote federal pediatric intensive care consultation center with endocrinologist involvement. These centers determine the sequence and timing of transfer of patients with DKA.

Transportation of children with DKA is not allowed:

1) until recovery of consciousness level of 10 or more points. Transportation may begin at a lower level of consciousness if deemed necessary by an intensive care specialist from a regional intensive care consultation center who has arrived at the scene;

2) if there is no possibility of intensive therapy (precise dosing of insulin, fluid therapy, device monitoring of vital body parameters) and blood glucose control during transportation of the child.

If admission to a pediatric intensive care unit is not possible, DKA should be managed in an adult intensive care unit [74].

Complications of Diabetic Ketoacidosis

Arrhythmias, infections, and kidney injury are all possible complications of DKA, but cerebral edema is the most serious and common. Cerebral edema is responsible for the vast majority of DKA deaths. It is diagnosed using clinical criteria (Table 6).

Symptomatic cerebral edema is associated with increased systolic blood pressure and heart rate. Patients with cerebral edema are characterized by prolonged hospital stay and correction of acidosis [59].

Table 6. Diagnostic criteria for cerebral edema in diabetic ketoacidosis.

Criteria	
Diagnostic	— abnormal motor or verbal response to pain — decorticatie or decerebrate posturing — cranial nerve paresis (especially III, IV, VI) — abnormal neurogenic respiratory patterns («grunting», tachypnea, Cheyne-Stokes breathing, apnea).
Major	— impaired thinking, lethargy, changes in the level of consciousness — constant slowing of the heart rate (decrease by more than 20 in 1 min.), not associated with an increase in fluid volume or sleep — urinary incontinence
Minor	— vomiting — headache — drowsiness or difficulty waking up — diastolic blood pressure >90 mmHg — age <5 years

Note. Summarized from [78]. One diagnostic + two minor criteria or one major + two minor criteria have a sensitivity of 92%.

Neuroimaging is performed only at the start of treatment. Brain computed tomography is preferable [45].

For early diagnosis of cerebral edema in DKA, point-of-care ultrasonography with measurement of optic nerve sheath diameter over time is recommended. This parameter is measured 3 mm posterior to the eyeball in the anterior axial transbulbar position. The transverse and vertical diameters of the eyeball are also measured and the ratios of optic nerve sheath diameter to transverse diameter and optic nerve sheath diameter to vertical diameter are calculated. These parameters should decrease with treatment, indicating reduction of optic nerve edema. The values of the optic nerve sheath diameter more than 4.5 mm, the ratio of the optic nerve sheath diameter to the transverse diameter more than 0.22 and to the vertical diameter more than 0.29 are considered abnormal [75].

Another method of assessing the severity of cerebral edema in DKA is the ratio of neutrophils to lymphocytes. This ratio is 2.82 (2.28–4.23) in children with DKA without cerebral edema, 5.66 (3.95–7.88) in those with subclinical edema, and 8.60 (4.73–12.17) in those with clinical manifestations of cerebral edema (P<0.001) [59].

The following methods are used to treat cerebral edema.

1. Hyperosmolar solutions:

a) mannitol 0.5–1 g/kg intravenous (i. v.) drip for 10–15 min [76]. The effect develops in 15 min, its duration is 120 min. The repeated dose is administered in 30 min. Contraindicated in hypernatremia (>165 mmol/L) [45].

b) hypertonic saline 3% 2.5–5 ml/kg i. v. over 10–15 min. May be an alternative to mannitol [77] but is associated with higher mortality [78]. At the same time, the use of cerebral oximetry along with administration of 3% hypertonic saline may improve outcomes in DKA and cerebral edema [79].

2. Patient positioning:

a) head end elevation at 30°;

b) centering the head on the midline;

c) «sniffing» position of the head;

d) lowering the feet end of the bed.

3. Tracheal intubation and lung ventilation. It is used when the level of consciousness is <9 points on the Glasgow Coma Scale.

When selecting the initial parameters of the ventilator, it is necessary that the PCO_2 after the patient is placed on the ventilator should be the same as before the tracheal intubation, in order to avoid the increase of acidosis and cerebral edema.

Normalization of pCO_2 should proceed in parallel with normalization of BE and pH.

Monitoring

Recommendation. Blood glucose should be measured every hour for the first 6 hours, then every 2 hours if blood glucose is falling steadily [45].

Recommendation. ABB should be checked at least once every 6 hours. In initially severe DKA, the first check should be performed within 3 hours of starting therapy [45].

In children with DKA on admission and every 2 hours of treatment in the ICU, the minimum necessary monitoring includes calculation and assessment of fluid balance, measurement of blood glucose, sodium, and potassium. Every hour the level of consciousness (alertness) is assessed, respiratory rate, heart rate, blood pressure (systolic, diastolic, mean), arterial saturation (SpO_2), ECG for changes in the T wave (hypokalemia below 3 mmol/L may cause its flattening or inversion) should be constantly monitored [45].

The high prognostic value of the blood urea nitrogen/albumin ratio in predicting the likelihood of death in DKA has been demonstrated [80].

Rehabilitation

Rehabilitation follows the basic principles of rehabilitating pediatric patients who have experienced critical illness and children with T1DM. If neurological deficits develop as a result of cerebral edema, a comprehensive rehabilitation program that involves a team of specialists is necessary.

Conclusion

DKA is a significant issue because it is associated with a comparatively high mortality rate in children, primarily due to the development of cerebral edema. Based on the integration of Russian and international best practices, we have developed guidelines for the diagnosis of DKA, its major complications, and the management of children with T1DM and DKA. Adherence to the diagnostic criteria for DKA, the use of short-acting insulin agents in combination with regular monitoring of blood glucose levels, the correction of dehydration with the appropriate use of glucose solutions based on the glycemic level, the prevention and early detection of signs of cerebral edema and its timely treatment can prevent poor outcomes in DKA.

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Asphyxial Circulatory Arrest with a Complex of Resuscitation Measures in an Experimental Model

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Summary

The majority of asphyxial circulatory arrest (CA) models have a number of disadvantages, such as the lack of uniform criteria for fixing CA and recovery of spontaneous circulation, short duration of CA episode and limited volume of post-resuscitation intensive care, poor similarity with resuscitation measures in current clinical anesthesiology/intensive care settings.

The aim of the study: to improve the experimental model of asphyxial CA by standardizing experimental procedures and using a complex of resuscitation measures replicating current CA management in clinical anesthesiology-intensive care.

Materials and methods. The experiments were conducted on 34 male Wistar rats, distributed into 2 groups: Group I included animals subjected to sham procedure (SP, *N*=12) and Group II — animals subjected to asphyxial circulatory arrest (CA, *N*=22) and subsequent resuscitation. Asphyxia in anesthetized rats was induced by rocuronium bromide injection, followed by recording of electrocardiogram (ECG), parameters of invasive blood pressure (BP) measurement and laser Doppler flowmetry (LDF) to assess skin perfusion. CA episode was maintained for 2 min, followed by a series of resuscitation measures and intensive therapy for 2 h. Circulatory parameters (ECG, BP, LDF), gas composition and arterial blood acid-base state (ABS) dynamics were evaluated.

Results. Monitored parameters were comparable in both groups at baseline after stabilization period. After exclusion criteria were applied 11 animals from SP group and 18 — from CA were included in the analysis. Tachycardia (heart rate, beats/min^{-1}, SP vs CA) was documented in the CA group: 218 [205; 236] vs 286 [272; 305], $P \leqslant 0.0001$), as well as recovery of skin perfusion to subnormal parameters in the first minutes after successful resuscitation. At minute 10 in the post-resuscitation period worsening of skin perfusion (M, perfusion units, SP vs CA): 14.7 [12.1; 16.5] vs 10.1 [7.0; 12.5], $P=0.0014$), and decompensated mixed acidosis (pH, SP vs CA): 7.42 [7.40; 7.43] vs 7.20 [7.13; 7.23], $P \leqslant 0.0001$) were documented in the CA group, however BP values were comparable (BP, mmHg, SP vs CA): 60 [58; 72] vs 67 [62; 82], $P=0.482$). At minute 120 post-resuscitation and at the end of intensive care period, both groups demonstrated similar values of the monitored parameters. Three out of 18 animals in the CA group died after resuscitation.

Conclusion. Electromechanical dissociation underlies CA in rats subjected to asphyxia. The use of LDF to assess peripheral blood flow makes it possible to standardize the severity of ischemic reperfusion injuries and improve reproducibility of the model. Series of resuscitation measures in experimental setting is justified from a bioethical point of view, and makes it possible to improve repeatability of preclinical research results in clinical practice.

Keywords: circulatory arrest; asphyxia; resuscitation measures; experimental model; rat

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

Introduction

Cardiac arrest (CA) is a critical condition in which there is no effective circulation. It is generally divided into out-of-hospital and in-hospital cardiac arrest. According to the European Resuscitation Council, the incidence of out-of-hospital CA in Europe is 67–170 cases per 100,000 population per year, and the survival rate of these cases (at hospital discharge) is approximately 8% [1]. The proportion of out-of-hospital CA in the total number of cardiac arrests is between 45% and 55% [2]. In-hospital cardiac arrest can occur in any hospitalized patient.

According to L. W. Andersen et al, the number of cases of in-hospital CA in the USA reaches 290,000 per year. The most common causes of CA are cardiac (50–60%), while the incidence of CA due to respiratory failure varies from 15% to 40% [3].

Currently, despite the development of new medical technologies, improvements in resuscitation protocols, and supportive therapy in the post-resuscitation period, the disability and mortality from sudden cardiac arrest remain dismal [4] and have not changed much over the past decade (10.4%) [5]. Thus, according to Patel et al, the outcome of in-hospital CA is highly variable depending on the medical care provided, with in-hospital survival of CA ranging from 45% to 85% and one-year survival varying from 5% to 35% [6].

Based on etiology, CA is classified as primary, which is due to cardiac causes, and secondary, which develops because of extracardiac factors [7]. In most cases, primary CA is caused by ventricular fibrillation (VF) or pulseless ventricular tachycardia (VT), for which electrical cardiac defibrillation is effective [8].

Secondary CA is most commonly caused by progressive respiratory and circulatory hypoxia, severe metabolic derangements (acidosis, hypo- and hyperkalemia), hypothermia, tension pneumothorax, cardiac tamponade, and intoxication, all of which are considered «irreversible» and require emergency treatment [9]. Asphyxial CA develops due to impaired gas exchange resulting in severe hypoxemia, hypercapnia, and tissue hypoxia. Respiratory acidosis and hypoxia damage the sinoatrial node and cardiac conduction system, resulting in bradyarrhythmias. If asphyxia persists and gas exchange is impaired, bradycardia progresses to electromechanical dissociation (EMD) or asystole [10, 11].

Experimental models of asphyxial CA in laboratory animals more closely resemble the mechanisms of CA in pediatric practice and in patients with acute respiratory failure of various etiologies [12]. Katz et al. developed one of the first such models in 1995 [13]. Disconnection of a halothane anesthetized experimental animal from the ventilator under neuromuscular blockade induced asphyxial CA. CA was diagnosed when the blood pressure dropped below 10 mmHg. Resuscitation procedures reflected current clinical approaches at the time. This model was later updated with different anesthetics, durations of asphyxia, and changes in the range of resuscitation procedures [14, 15].

There are several common drawbacks to the experimental models of asphyxial CA described here. The lack of a standardized approach to anesthetic selection may have an indirect effect on macro- and microhemodynamic parameters, leading to biased data [16]. The duration of CA varies depending on the individual characteristics of the experimental animal and this has a direct effect on the success of resuscitation. For example, if CA lasts 7 minutes, the chance of restoring spontaneous circulation is less than 50% [17]. Other drawbacks include the lack of uniform and clear criteria for recording arrest and return of spontaneous circulation, the short duration (30–120 minutes) and limited volume of intensive care after resuscitation, and the low consistency with actual resuscitation practice in current clinical anesthesiology and intensive care.

The aim of this study was to improve the experimental model of asphyxial CA by standardizing the experimental procedures and applying a set of resuscitation measures similar to those used in patients with CA in current clinical anesthesiology and intensive care.

Materials and Methods

A prospective randomized controlled experimental study (in vivo) was conducted at the V. A. Negovsky Research Institute of General Reanimatology, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology (FRCCICMR), Moscow, Russia, on 34 adult male Wistar rats weighing 250–350 g. The animals were divided into two groups: group 1, sham-operated animals (SO group, *N*=12); and group 2, animals with asphyxial CA and subsequent resuscitation (CA group, *N*=22). Animals were deprived of food but had free access to water for 12 h prior to the experiment.

The study was conducted in accordance with accepted national and international bioethical standards (Directive 2010/63/EU). The study protocol was approved by the Local Ethics Committee of the FRCCICMR (Protocol No. 4/21/7, dated September 29, 2021).

The following exclusion criteria were used: serious complications or death during the experiment before induction of cardiac arrest (side effects of anesthesia, complications of the surgical manipulations performed) and achievement of a humane endpoint of the study (severe trauma, pain and suffering of the animal that cannot be alleviated by the available means).

Anesthesia and surgical manipulations. All animals included in the experiment were anesthetized with a combination of tiletamine/zolazepam («Zoletil 100», Virbac) 20 mg/kg + xylazine («Xylanit», LLC «NITA-FARM», Russia) 5 mg/kg intraperitoneally with additional administration of Zoletil 10 mg/kg at the first signs of animal arousal.

The left carotid artery and the left internal jugular vein were catheterized with a PE-50 polyethylene catheter (OD 0.95 mm, ID 0.58 mm, SciCat, Russia) for the purpose of invasive blood pressure (BP) measurement and arterial blood sampling, drug administration, and implementation of

the postresuscitation intensive care protocol, according to the previously described method [18]. If necessary, the catheter was flushed with 0.1–0.2 ml of unfractionated heparin solution (20 U/mL) to maintain patency.

Tracheal intubation and ventilation. To ensure adequate ventilation during preparation and resuscitation, the trachea was intubated via direct laryngoscopy using a 16G venous catheter. After neuromuscular blockade by intravenous administration of rocuronium bromide 1.4 mg/kg body weight, ventilation was performed with the SAR-1000 (CWE Inc., USA) in CMV/VC mode. The tidal volume (V_t) was calculated according to the nomogram in the ventilator manual (approximately 0.7 ml per 100 g body weight), with an oxygen fraction (FiO_2) of 21%, a respiratory rate (f) of 60/min, and an inspiration/expiration ratio (I:E) of 1:2. If spontaneous breathing persisted, a repeated dose of rocuronium bromide was administered.

Preparatory measures. Rats were restrained in the supine position on a heated MouseMonitor S monitor platform (INDUS Instruments, USA) (Fig. 1). A rectal thermometer was used to measure and control central body temperature. The target central body temperature was 36.0–37.0°C. To prevent heat loss, the animal was covered with insulating material. The animals were allowed to stabilize for 15–20 min before measurements were started.

BP measurement. The arterial catheter was connected to a Deltran DPT-100 transducer (Utah Medical Products, USA) via a Y-piece and infusion line. The analog BP signal from the transducer and BP-100 device (CWE Inc., USA) was transmitted to a PowerLab16/35 device (ADInstruments, Australia) connected to a personal computer (PC). The digitized BP signal was recorded, stored in the PC's hard disk memory, and analyzed using LabChart Pro 8 software. The mean arterial pressure (BPmean) for the measurement period (5 min) was calculated from the BP curve data.

ECG registration. The analog ECG signal from the surface electrodes of the MouseMonitor S platform (INDUS Instruments, USA) was transmitted to a PowerLab16/35 device (ADInstruments, Australia) connected to a PC. The digitized ECG signal in three standard leads (I, II, III) was recorded, stored in the PC hard disk memory and analyzed using LabChart Pro 8 software. The average heart rate (HR) for the measurement period (5 min) was calculated from the ECG data.

Arterial blood gas and acid-base balance (ABB). Arterial blood (0.2 ml) was withdrawn from the arterial catheter into an «insulin» syringe (1.0 ml) after prior aspiration of the residual flushing solution from the catheter. The syringe walls were pretreated with unfractionated heparin 5,000 U/mL (no more than 50 µL). Arterial blood gases and ABB (pH, pCO_2, pO_2, BE, HCO_3, SaO_2, and lactate levels) were analyzed using CG4+ reagent cartridges for the iSTAT 1 analyzer (Abbott Point of Care Inc., USA).

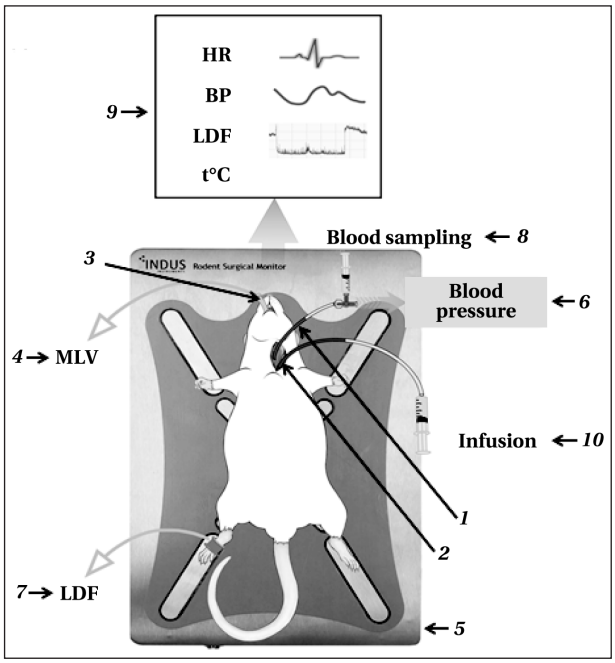

Fig. 1. Schematic of the asphyxial cardiac arrest modeling experiment.
Note. *1* — arterial catheter; *2* — venous catheter; *3* — intubation tube; *4* — MLV (mechanical lung ventilation) using ventilator for small laboratory animals; *5* — heated platform of the monitor of functional parameters of small laboratory animals; *6* — device for direct measurement of blood pressure; *7* — LDF (laser Doppler flowmetry) device; *8* — sample of arterial blood for assessment of blood gases and acid-base status; *9* — graphic representation of recorded physiological parameters (ECG, blood pressure, body temperature, LDF); *10* — syringe-doser for continuous intravenous infusion in the post-resuscitation period.

Registration of skin perfusion by laser Doppler flowmetry (LDF). The right hind paw of the rat was wiped with a wet gauze cloth to clean the skin surface. The optical probe of the LAZMA MC-3 device (LLC SPE «LAZMA», Russia) was placed perpendicular to the central part of the plantar surface of the animal's foot. To fix the probe tip to the skin surface, a strip of soft adhesive plaster was wrapped around the tip and the paw of the animal. LDF registration took 5 minutes. LDF software version 3.2.0.475 (OOO NPP «LAZMA», Russia) was used to calculate the mean perfusion value *(M)* measured in conventional perfusion units (pfu).

Model of asphyxial cardiac arrest and resuscitation. After registration of baseline parameters (Fig. 2), we modeled CA according to a previously described method [19] with the author's modifications. The animal was reinjected with myorelaxant (rocuronium bromide 1.4 mg/kg) and the breathing circuit of the ventilator was disconnected (Fig. 3). ECG, BP, and LDF monitoring was continued to determine the time of CA. When mean BP (BP_{mean}) decreased to 20 mmHg in combination with marked bradycardia and skin perfusion decreased to the

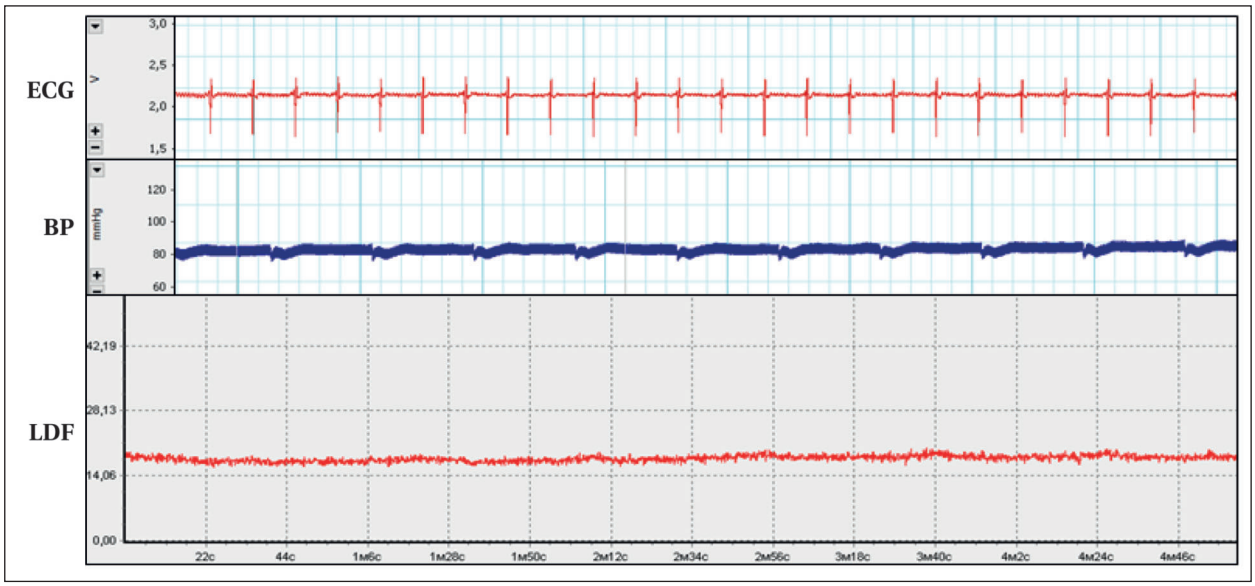

Fig. 2. Recorded blood pressure (BP), electrocardiogram (ECG), and laser Doppler flowmetry (LDF) signals in the anesthetized rat at baseline (before induction of asphyxia).

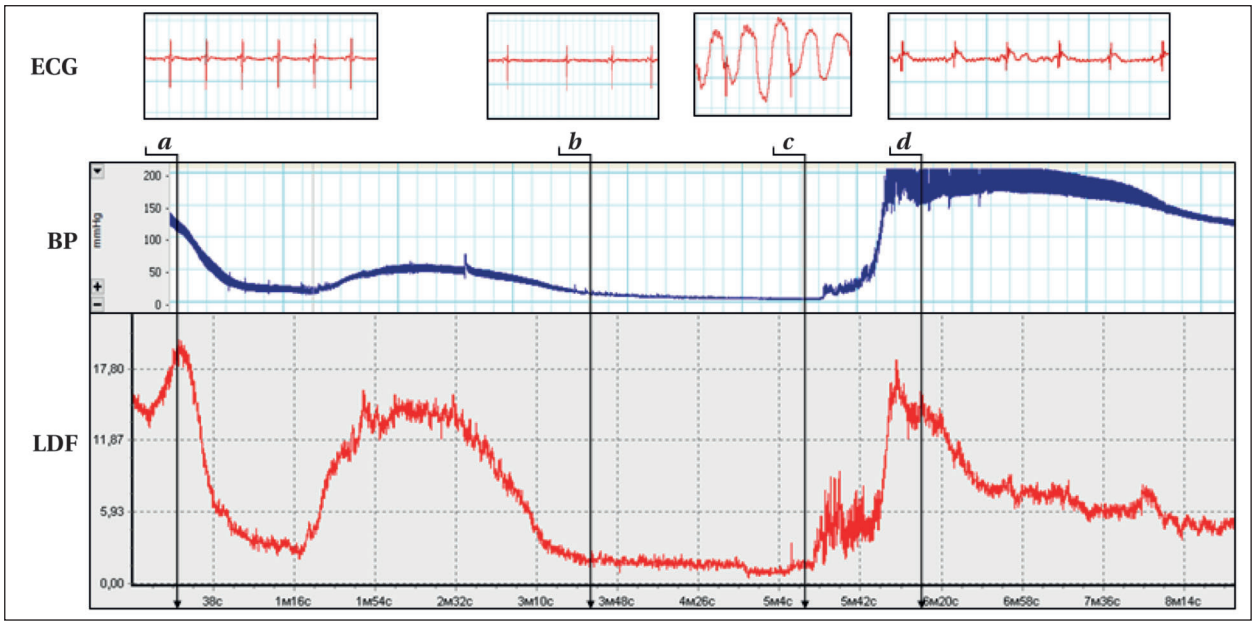

Fig. 3. Changes in ECG, BP and LDF parameters during induction of asphyxia, cardiac arrest and resuscitation.
Note. *a* — induction of asphyxia; *b* — onset of cardiac arrest; *c* — onset of resuscitation; *d* — return of spontaneous circulation. In the above example, at the 2nd minute of asphyxia, a transient increase in blood pressure and skin perfusion was recorded in the anesthetized animal, preceded by a marked decrease (agonal period).

level of LDF equal to «biological zero» (3–4 pfu), it was considered that there was no effective tissue perfusion, and the onset of cardiac arrest was recorded (Fig. 3). Resuscitation was initiated 2 minutes after CA (Fig. 3). Mechanical ventilation was resumed in CMV/VC mode with the following parameters: FiO_2=100%, f=80/min, I:E=1:2, and V_t according to the rat nomogram. Chest compressions were performed in an anteroposterior direction in the supine position at a rate of 200/min, the depth of compression was 1/3 of the anteroposterior size of the chest, followed by complete decompression of the chest. Adrenaline 0.005 mg/kg was then administered intravenously.

After one minute of chest compressions, chest compressions were stopped and heart rate, mean blood pressure, and skin perfusion were assessed. If CA persisted, resuscitation was continued with cardiac rhythm assessment every minute. Repeated administration of 0.005 mg/kg epinephrine was performed every 5 minutes of resuscitation. ECG, BP, and LDF monitoring continued. If resuscitation was ineffective within 10 minutes, it was stopped. When spontaneous circulation was restored (increase

in BP_{mean} above 50 mmHg, increase in skin perfusion above the «biological zero» of LDF (Fig. 3)), lung ventilation with oxygen was continued along with monitoring of BP, ECG, LDF, infusion of NaCl 0.9% solution 10 mL/kg/h.

Blood gases and ABB were measured 5 minutes after resuscitation and the results were used to adjust the ventilatory parameters. In cases of severe metabolic acidosis (pH <7.2, BE <–10 mmol/L), infusion of 4% $NaHCO_3$ 1 mmol/kg was performed. Two hours after resuscitation (after completion of measurements), a spontaneous breathing test was performed, the breathing circuit was disconnected from the endotracheal tube, and spontaneous breathing attempts were recorded for 2 minutes.

Study time points. The parameters studied were recorded at baseline after the stabilization period (time point 1), at 5–10 min (time point 2), and at 115–120 min (time point 3) after return of spontaneous circulation. Animals in the SO group underwent the same measurements and procedures as those in the CA group, except for induction of asphyxia, CA, and resuscitation (chest compressions, administration of adrenaline, ventilation with FiO_2 of 100%).

Data processing and statistical analysis. The parameters measured during the experiment were recorded in the experimental flow chart (body weight, body temperature, arterial blood ABS) and in the PC hard disk memory (ECG, BP, LDF) using the appropriate software. At the end of the experiment, the primary data were analyzed and the studied parameters were calculated. The sample size was calculated in the StatMate 2.0 program (GraphPad Software, USA) based on the previous series of experiments on rat skin microcirculation, taking into account the variability of skin perfusion indices (according to LDF data), the estimated mortality in the CA group of about 30%, and the power of the method of more than 0.9. The M value used in the sample calculation was 16.22 pfU, and the standard deviation was 2.22 pfU. The results were analyzed using the software packages Statistica 13.0 (StatSoft, USA) and Prism 8 (GraphPad Software, USA). Because most of the parameters studied had a non-normal distribution (based on the Shapiro–Wilk test), the Mann–Whitney *U*-test was used to assess the significance of differences between groups, and the Friedman test was used to assess the change in the index within group (for pairwise comparisons, the Wilcoxon test with Bonferroni correction was used). Results are presented as median and interquartile range *Me* [25%; 75%]. Differences were considered significant when $P<0.05$.

Results and Discussion

According to the exclusion criteria, one animal was excluded from the SO group (N=12) and four from the CA group (N=22). Thus, 11 animals from the SO group and 18 from the CA group were used in the subsequent analysis. There were no deaths in the SO group, whereas in the CA group, 3 out of 18 animals (16.7%) died after resuscitation.

In the CA group, the time from induction of asphyxia to the moment when mean blood pressure fell below 20 mmHg in combination with marked bradycardia and reduction of skin perfusion to the level of LDF «biological zero» (3–4 pfU) was evaluated (CA documentation). The mean time from induction of asphyxia to CA documentation was 220 seconds [180; 255], and the total time from induction of asphyxia to return of spontaneous circulation was 330 seconds [300; 375] in the CA group. After induction of asphyxia, one third of the animals in the CA group showed a transient decrease in BP below 20 mm Hg, a decrease in M according to LDF to the level of «biological zero» with a gradual return to subnormal values, and no pathological rhythms on the ECG. These episodes were considered the agonal period, and the time between the induction of asphyxia and the documentation of AC was prolonged.

During the first minutes of return of spontaneous circulation in the CA group, the following changes in the studied functional parameters were observed: arterial hypertension up to 200/160 mmHg, increase in M to subnormal values, and sinus tachycardia. At baseline, there was no difference between the groups in any of the parameters studied (Table). Ten minutes after resuscitation and 2 hours after return of spontaneous circulation, there was no statistical difference in BP_{mean} between groups. Ten minutes after resuscitation, the CA group had a significantly higher HR values than the SO group (Table). The CA group had a lower mean skin perfusion (M) at the 10th minute of the postresuscitation period, but there was no difference between the groups at the 120th minute (Table).

Animals in the CA group developed pronounced hypercapnia, hyperlactatemia, and an increase in base deficit as well as decompensated mixed acidosis by the 10th minute of the post-resuscitation period. Furthermore, hyperoxemia was detected in animals from the CA group compared to the SO group with the mechanical ventilation with 100% oxygen fraction, while the oxygenation index (p/F) was reduced (Table). Two hours after return of spontaneous circulation, there was no statistical difference in ABB or blood gases between the groups (except for higher blood oxygenation in the CA group), demonstrating the efficacy of intensive therapy and mechanical ventilation in the CA group (Table). All surviving animals in the CA group showed spontaneous respiration 2 h after blood circulation was restored after cessation of mechanical ventilation, with underlying depressed consciousness.

Experimental models of asphyxial CA in laboratory animals better reproduce the mechanisms

Table. Circulation parameters. blood gas and acid-base balance of rats at baseline and in the early post-resuscitation period; *Me (LQ; UQ)*.

Parameter	Baseline			5–10 min of the post-resuscitation period			115–120 min of the post-resuscitation period		
	SO group, *N*=11	CA group, *N*=18	*P*-value	SO group, *N*=11	CA group, *N*=18	*P*-value	SO group, *N*=11	CA group, *N*=15	*P*-value
BP_{mean}, mmHg	70 [65; 78]	72 [65; 77]	0.912	60 [58; 72]	67 [62; 82]	0.482	63 [57; 68]	63# [62; 67]	0.892
HR, per minute	222 [210; 231]	238 [217; 253]	0.159	218 [205; 236]	286 [272; 305]	0.0001	232 [206; 257]	247 [236; 261]	0.055
M, pfu	14.8 [13.0; 16.5]	15.9 [13.4; 17.4]	0.610	14.7 [12.1; 16.5]	10.1# [7.0; 12.5]	0.0014	15.4 [13.0; 17.4]	14.9 [13.2; 16.1]	0.443
pH	7.41 [7.38; 7.43]	7.43 [7.40; 7.50]	0.674	7.42 [7.40; 7.43]	7.20# [7.13; 7.23]	<0.0001	7.44 [7.40; 7.46]	7.47 [7.33; 7.53]	0.437
$PaCO_2$, mmHg	37.8 [34.1; 41.3]	38.4 [34.7; 41.9]	0.991	37.8 [32.7; 42.4]	51.3# [41.2; 60.9]	0.0001	34.7 [31.5; 39.7]	33.9# [30.3; 52.9]	0.861
PaO_2, mmHg	70 [59; 86]	69 [63; 79]	0.851	78 [64; 91]	120# [83; 139]	0.0045	82 [75; 91]	291# [194; 376]	0.0001
BE, mmol/L	0 [–1; 1]	1 [–1.5; 3]	0.554	–0.5 [–2.7; 1.7]	–9# [–11; –8]	<0.0001	–1.0 [–2.0; 0.5]	–2.0 [–4.0; 3.0]	0.965
Lactate, mmol/L	1.16 [1.02; 1.53]	1.16 [0.88; 1.65]	0.782	1.13 [1.52; 5.42]	5.58# [4.65; 6.90]	<0.0001	1.21 [0.95; 1.94]	1.47 [1.16; 2.57]	0.473
SaO_2. %	93 [90; 97]	94 [92; 96]	0.974	95 [93; 97]	98 [93; 99]	0.087	96 [95; 97]	100# [99; 100]	0.0001
HCO_3, mmol/L	24.2 [23.4; 25.4]	25.4 [23.1; 26.9]	0.588	24.2 [17.1; 25.6]	18.4# [17.2; 20.8]	0.0002	23.3 [20.3; 24.6]	24.3# [21.0; 25.8]	0.650
p/F	333 [297; 404]	337 [314; 385]	0.991	337 [285; 390]	121# [93; 136]	<0.0001	390 [358; 425]	295# [202; 359]	0.0338

Note. BP_{mean} — mean blood pressure; HR — heart rate; M — mean perfusion value; $PaCO_2$ — partial pressure of CO_2 in the arterial blood; PaO_2 — partial pressure of O_2 in the arterial blood; BE — base excess; SaO_2 — oxygen saturation of arterial blood; HCO_2 — level of bicarbonate; p/F — oxygenation index. *P* — exact *P*-value for SO vs CA; # — $P<0.05$ vs baseline (with Bonferroni correction for multiple comparisons).

of cardiac arrest due to extracardiac causes in patients with different profiles and are increasingly used in modern experimental medicine [12].

The literature search revealed a number of methodological solutions for modeling asphyxial cardiac arrest, as well as the identification and evaluation of their advantages and disadvantages. Anesthetic agents include both inhalational (sevoflurane and isoflurane) [20, 21] and non-inhalational (pentobarbital and chloral hydrate) [15, 22]. When inhalational anesthetics are used, anesthesia is discontinued before asphyxia is induced. Methodologically, this reduces the effect of anesthesia on the pathogenesis of ischemia-reperfusion injury as a component of multiorgan dysfunction [23], but it does not reflect the pathogenesis of CA in clinical anesthesiology.

In this study, we used a combination of the NMDA receptor antagonist tiletamine/zolazepam and the central α2-adrenoreceptor agonist xylazine (veterinary analog of dexmedetomidine) to achieve minimal cardiorespiratory effects, sufficient depth of anesthesia, and myorelaxation for invasive manipulations [16].

Despite the similarities in the methods used by different authors to model asphyxial CA, a review of literature sources reveals a significant heterogeneity of models, implying insufficient comparability and reproducibility of the obtained experimental data [24].

In general, an evaluation of the methodological approaches used in existing models allows us to identify a number of common shortcomings. Most experimental models use specific BP values to calculate the moment of CA, which vary widely between sources (from 10 mm Hg to 30 mm Hg) [15, 25]. At the same time, the authors do not assess organ and tissue perfusion, and the duration of CA is often determined by the total duration of asphyxia rather than CA itself, which reduces standardization of the severity of ischemia-reperfusion injury.

To confirm the time of cardiac arrest and return of spontaneous circulation after resuscitation, we used the LDF method to assess skin perfusion in conjunction with ECG and blood pressure monitoring. The duration of cardiac arrest was measured from the moment of EMD using the following criteria: BP_{mean} drop below 20 mmHg for at least 10 seconds, marked bradycardia (less than 100/min in rats) or other agonal heart rhythm (idioventricular rhythm, blocks, etc.), and skin perfusion at the «biological zero» LDF level.

In various studies, supportive therapy in the post-resuscitation period was limited in time and volume and did not correspond to modern resuscitation protocols (insufficient duration of ventilation, lack of clear protocols for the use of vasopressors and inotropes, lack of protocols for volemic support and ABB correction, etc.). These features limit the translational potential of preclinical study results for clinical anesthesiology and resuscitation [26–27]. When we performed supportive intensive therapy in the post-resuscitation period of the study, the

BPmean values did not show statistical differences between the groups at either 10 or 120 minutes, and the mixed acidosis observed in the CA group was compensated by 120 minutes, indicating the effectiveness of intensive therapy.

Thus, the results of the experiments showed that the use of prolonged non-inhalational combined anesthesia, documentation of CA onset and restoration of spontaneous circulation using LDF, performing comprehensive intensive care in the post-resuscitation period for at least 2 hours, improved the quality of modeling of asphyxial CA by reducing the variability of functional parameters of animals, improving the reproducibility of the model and its similarity to the post-resuscitation disease in humans in the clinical setting. This improvement was achieved using methodological techniques described in the literature. In particular, we considered the experience of modeling asphyxial CA with resuscitation to evaluate the neuroprotective effects of hypothermia and glibenclamide as described by Huang et al. [17].

A limitation of this study is the use of non-inhalational anesthetics to induce anesthesia. Despite several advantages over inhalational anesthetics (low severity of cardiorespiratory manifestations, sufficient depth of sedation and myorelaxation for surgical manipulations, and a sufficient degree of analgesia in the experimental animal), the use of combined anesthesia reduces the similarity of the model to out-of-hospital CA and may also contribute to the bias of experimental data from the perspective of drug property studies. It is also necessary to consider the toxic effects of oxygen and to limit the duration of high fractional oxygen ventilation [28]. Furthermore, the modeling of asphyxial CA in the current variant is a technically demanding process that requires both the use of specialized equipment and the development of appropriate investigator skills. The study of remote (3–7 days and longer) outcomes of post-resuscitation disease in experimental animals with longer duration of CA (4–8 min) is of great clinical importance, but raises additional organizational (need for prolonged intensive care) and bioethical concerns.

Conclusion

Cardiac arrest in rats during asphyxia under general anesthesia occurs via electromechanical dissociation. Separate documentation of the time of respiratory and cardiac arrest and using LDF to assess peripheral blood flow allows standardization of the severity of ischemia-reperfusion injury and improves model reproducibility. The use of a set of resuscitation measures that fit modern standards of patient management in clinical anesthesiology and intensive care is bioethically sound and allows for better translation of preclinical study results into clinical medicine.

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Neuroprotection by Anesthetics in Brain Injury Models

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Summary

The aim of the study was to compare the effect of sevoflurane and chloral hydrate on the neurological status and volume of brain damage after trauma and ischemia in experimental models of traumatic brain injury (TBI) and focal ischemic stroke (IS) induced by photothrombosis (PT).

Materials and methods. The experiments were performed on mongrel Wistar rats weighing 250–300 g (*N*=43). There were 4 groups: the Ischemia + Sevoflurane group (IS_S) (*N*=10), the Ischemia + Chloral hydrate group (IS_{CH}) (*N*=10), TBI + Sevoflurane group (TBI_S) (*N*=13), and TBI+Chloral hydrate group (TBI_{CH}) (*N*=10). Ischemic brain damage was modelled using Rose Bengal (RB) dye-induced PT, and TBI was modelled using mechanical force-induced concussion.

Results. MRI findings indicate lower volumes of brain damage (mm^3) in rats from TBI_S group compared with the TBI_{CH} group (19±5 vs. 60±5, *P*<0.0001), and in the IS_S group compared with the IS_{CH} group (9.8±1.5 vs. 21.5±2, *P*=0.0016). Moreover, there was a significant difference between IS_S and IS_{CH} groups based on the protocol assessment of neurological status on day 14 with higher scores in IS_S (11.4±1.8 vs. 4.9±2.6, *P*<0.0001).

Conclusion. Taking into account the data obtained, we recommend a careful choice of anesthesia when modeling ischemic stroke and traumatic brain injury in animals. In particular, the neuroprotective effect of sevoflurane should be taken into account in the PT and TBI models.

Keywords: neuroprotection; anesthetics; photoinduced ischemic stroke; TBI model; brain injury models; sevoflurane; chloral hydrate

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

Introduction

Models of ischemic injury, such as photochemically induced thrombosis, brain injury, middle cerebral artery occlusion, and others, are most commonly used to evaluate the neuroprotective properties of drugs. The models of dosed open brain contusion injury [1] and photochemically induced thrombosis have proven to be relevant and have a number of advantages, including low invasiveness, high reproducibility, low mortality, and the ability to control the extent of brain damage [2, 3]. To evaluate the effect of different therapeutic agents in brain injury models, it is necessary to eliminate the influence of various «confounding» factors, such as the anesthetic method or animal heterogeneity.

Anesthetics used in different experimental models possess neuroprotective properties, which impede assessing the neuroprotective effects of different therapeutic agents.

Therefore, the aim of our study was to compare the effects of sevoflurane and chloral hydrate on the neurological status and extent of brain damage in photochemically induced thrombosis (PIT) and traumatic brain injury (TBI).

Materials and Methods

Animals. Experiments were performed on 43 Wistar crossbred rats, weighing 250–300 g, maintained in a vivarium with a 12/12-hour light/dark cycle at a constant temperature (22±2°C). Animals were used in experiments in accordance with the Animal Care Protocol approved by the Animal Ethics Committee of A. N. Belozersky Research Institute of Physicochemical Biology, protocol No. 2/20, February 12, 2020.

The following four groups were distinguished:

- Ischemic stroke + sevoflurane (IS_S) group (*N*=10)
- Ischemic stroke + chloral hydrate (IS_{CH}) group (*N*=10)
- TBI+Sevoflurane (TBI_S) group (*N*=13)
- TBI+chloral hydrate (TBI_{CH}) group (*N*=10).

Anesthesia. In the IS_{CH} and TBI_{CH} groups, rats were anesthetized with chloral hydrate (300 mg/kg,

intraperitoneally). In the IS_S and TBI_S groups, animals were anesthetized with sevoflurane (5% in a gas mixture with oxygen at a flow rate of 2 L/min), and anesthesia was maintained with 2–3% sevoflurane at a flow rate of 2 L/min through a mask. Rats were placed under an infrared heating lamp for 1 h before emergence from chloral hydrate anesthesia. The duration of sevoflurane inhalation was 39.4±3.4 min and 25.5±5.4 min in the IS_S and TBI_S groups, respectively. The body temperature of the rats was maintained at 37.0±0.5°C throughout the experiment. Thermometry was performed by installing a rectal body temperature sensor, and thermoregulation was maintained in automatic mode by connecting the heating module to a thermostat and setting the limits.

PIS model. A previously described photochemical thrombosis protocol [2, 3] was used. Focal ischemic stroke was modeled in the sensorimotor cortex of the rat brain (stereotaxic coordinates from bregma: 0.5 mm distal and 2.5 mm lateral). The photosensitive dye rose Bengal was injected intravenously (3%, 40 mg/kg; Sigma-Aldrich, St. Louis, Missouri, USA). The exposed cranial region was irradiated with green light at λ=550 nm for 15 minutes.

TBI modeling. TBI modeling was performed according to the open brain contusion injury method [1].

Magnetic resonance imaging (MRI). The study was performed on day 14 after PIS and TBI on a 7 T magnetic field induction tomograph with a 105 mT/m gradient system (BioSpec 70/30, Bruker, Germany). Animals were anesthetized with isoflurane (1.5–2%) and placed in a positioning apparatus with a stereotaxic and thermoregulatory system (as described previously [4]).

A standard rat brain examination protocol was used, including acquisition of T2-weighted images [4]. The extent of brain damage was assessed by graphical analysis of the MR images and calculation of the volume of the damaged brain area in mm3 using ImageJ software (National Institutes of Health image software, Bethesda, MD, USA).

Limb placement test (LPT). Neurological status was assessed 3, 6, and 14 days after PIS and trauma. We used a well-known protocol based on a study by De Ryck et al. [5] and modified by Jolkkonen et al. [6]. The following scores were calculated for each task: 2 points, normal response; 1 point, delayed and/or incomplete response; and 0 points, no response. A total score was calculated for the seven tasks.

Statistical analysis of quantitative data was performed using GraphPad Prism 6 software (GraphPad Software). Normality of data distribution was tested using the Shapiro–Wilk test. The Student's *t*-test was used to compare the two groups if the samples compared had a normal distribution of variables. Otherwise, the Mann–Whitney test was used. Two-way ANOVA was used to compare two groups of animals at three different time points. Data are presented as *mean*±*SD*.

Results

We found a significant reduction in the extent of brain damage when sevoflurane was used as an anesthetic in a model of traumatic brain injury (Fig. *a*). MRI showed that it was almost 3 times larger with chloral hydrate (60±5 mm³) than with sevoflurane (19±5 mm³) ($P<0.0001$). There were no significant differences in neurological status between the groups (Fig. *b*).

In the PIS (ischemic stroke) model, we also observed a decrease in brain lesion when sevoflurane was used (Fig. *c*). In addition, the lesion volume (mm³) was 2-fold larger with chloral hydrate than with sevoflurane (21.5±2 vs. 9.8±1.5, $P=0.0016$). When the neurological status of rats in the IS_S and IS_{CH} groups was analyzed on day 14, significant differences in scores were found (11.4±1.8 vs. 4.9±2.6, $P<0.0001$) (Fig. *d*).

Discussion

Anesthesia used for various purposes, including clinical, veterinary, and research practice, should generally meet the following criteria: reversible loss of consciousness, akinesia, amnesia, and analgesia [7]. Data regarding the analgesic properties of chloral hydrate, a drug commonly used in animals, are conflicting. In recent years, these properties have been found to be similar to those of other commonly used anesthetics such as ketamine-xylazine, pentobarbital, and urethane [8]. In a 2023 review by Ward-Flanagan and Dickson, it was noted that chloral hydrate also produces analgesia at the cellular level through the action of its metabolite 2,2,2-trichloroethanol, which inhibits pain transmission in mammalian dorsal root ganglion neurons. In animals, this drug is most commonly administered intraperitoneally, although intravenous administration is also possible [8].

Inhalational anesthetics, including isoflurane, sevoflurane, and desflurane, are also commonly used in animals [9]. These agents are vaporized in special vaporizers, added to the carrier gas, and administered to the animals through the respiratory tract, providing rapid induction of anesthesia, short duration of action, and rapid clearance from the body after termination of supply, which is a distinct advantage over intraperitoneal anesthetics.

When studying brain injury and searching for neuroprotective drugs, it is important to use the absence of intrinsic damaging or neuroprotective effects as a criterion for the choice of anesthetic. In the presence of such properties, it is difficult to detect the proper action of the drug owing to its anesthetic effect.

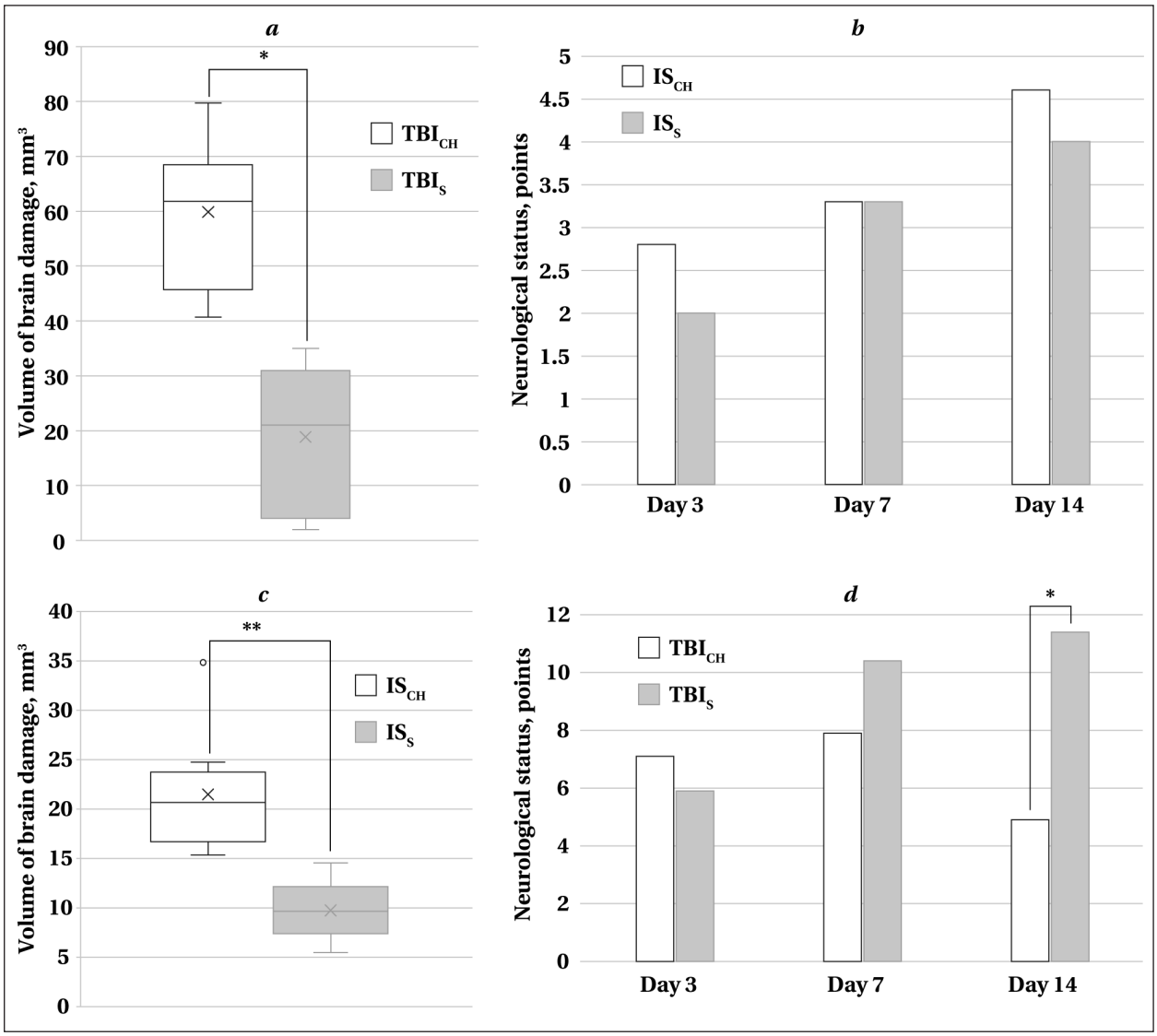

Fig. Comparison of study groups according to MRI data of the volume of brain damage *(a, c)* and scores of neurological status assessment *(b, d)*.
Note. Statistically significant differences: * — $P<0.0001$; ** — $P=0.0016$.

Literature shows that some anesthetics, such as dexmedetomidine [10] and zoletil [11], have neuroprotective properties.

In the model of PIS (ischemia) and TBI, we obtained data on the probable presence of anesthetic preconditioning properties of the anesthetic sevoflurane. Thus, sevoflurane reduced the volume of the damaged brain area in operated rats. This reduced the lesion volume observed on MRI in the group of animals operated under sevoflurane anesthesia in both models of brain injury (Fig. *a*, *c*).

In addition, the mean number of protocol scores for neurological status assessment was significantly greater in the IS_S group than in the IS_{CH} group ($P<0.0001$, Fig. *d*). Furthermore, only in the sevoflurane group were animals with maximum scores (3 rats out of 10). Although no improvement in neurological status was observed in rats treated with sevoflurane during traumatic injury in the limb placement test, the reduction in lesion size may affect the results of other neurological tests and the cellular response when studying the neuroprotective properties of drugs while using sevoflurane.

Given the data obtained, it can be concluded that it is difficult to separate the neuroprotective properties of the drugs studied from those of the anesthetic sevoflurane used in a model of TBI.

Several other studies have also described the neuroprotective effects of sevoflurane in a model of TBI [12]. TBI was induced in rats who were anesthetized with 3% sodium pentobarbital (50 mg/kg) and then received inhalation of sevoflurane for 1 h. Sevoflurane reduced brain edema, improved neurological parameters, and decreased neuronal apop-

tosis and autophagy in rats with TBI. Sevoflurane postconditioning activates fibroblast growth factor 2 (FGF2), which protects the blood-brain barrier from damage during TBI in mice [13].

There is also evidence that sevoflurane may exert neuroprotective effects in models of focal or global cerebral ischemia [14–17]. The neuroprotective effects of sevoflurane have been observed when administered by inhalation prior to ischemia modeling («preconditioning» effect) [18, 19]. For example, sevoflurane-induced preconditioning 15 min or 24 h before global cerebral ischemia reduced the extent of neuronal damage in rats [18]. In *in vitro* models, sevoflurane preconditioning of rats 15 min prior to hypoxia and reoxygenation dose-dependently increased the recovery of hippocampal neuronal function after hypoxia [19].

The effect of chloral hydrate on brain injury in rats in both models cannot be completely excluded. Studies have shown the effects of chloral hydrate preconditioning in a mouse model of ischemic stroke (middle cerebral artery occlusion) [20]. Chloral hydrate was shown to reduce the extent of damage and improve neurological status. A possible mechanism of action is an increase in the expression of annexin A1, an anti-inflammatory factor [20]. Chloral hydrate also had neuroprotective properties when used in combination with ischemic preconditioning [11, 21]. Despite these findings, sevoflurane was found to be more neuroprotective than chloral hydrate. However, the mechanism underlying this effect is not fully understood.

Conclusion

Sevoflurane is more neuroprotective than chloral hydrate in rat models of brain injury. The feasibility of using sevoflurane as an anesthetic agent when evaluating the neuroprotective potential of other therapeutic agents is questionable. Replacing sevoflurane with chloral hydrate may be a reasonable option.

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Nutritional and Metabolic Status Control and Nutritional Support in Patients with Pancreatic Sepsis (Review)

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Summary

Acute pancreatitis (AP) is associated with pancreonecrosis in 30% of patients, who may fall at 80% high risk of death when infected pancreatic necrosis progresses to sepsis. Given the catabolic nature of the disease and the significant influence of nutritional status on its course and outcome, these patients require an adequate nutritional support (NS) based on an adequate assessment and control of nutritional and metabolic status.

The aim of the study: to identify trends in developing new tools for assessment of nutritional and metabolic status, and provision of NS in patients with pancreatic sepsis (PS).

Materials and methods. Keyword search in the PubMed, Scopus and E-library databases for the period from 2018 to 2023 yielded 95 publications, of which 16 meta-analyses and 6 systematic reviews met the requirements.

Results. all existing to date scales for assessment of nutritional deficiency in patients with PS have low prognostic value. Of them, mNUTRIC scale seems to be the most appropriate assessment tool. Recommended by EPSEN guidelines tools to assess the risk of nutritional deficiency it is not suitable for ICU patients. Indirect calorimetry should be preferred vs routine calculation formulas in assessing patient's energy needs in case of PS. It was also found that «standard» anthropometric values, such as BMI, are not always informative and prognostically significant in patients with severe AP in the ICU. Analgesia, infusion therapy, as well as detection and correction of intraperitoneal hypertension are not only integral components of intensive care for PS but are indispensable for supplying adequate NS in PS patients. It was found that early enteral nutrition is the preferred method of NS, although questions concerning choice of tube insertion site, as well as all parameters of tube feeding remain unanswered. The optimal composition of enteral nutrition for patients with PS has not been established, which is indirectly confirmed by the variety of enteral mixtures available on the market. The refeeding syndrome that occurs at initiation of NS was characterized as a life-threatening condition.

Conclusion. NS, based on adequate assessment of disorders and control of the nutritional and metabolic status is an integral component of intensive care in PS patients. It can reduce the probability and number of potential complications, time of stay in the ICU, cost of treatment, and improve patient's prognosis.

Keywords: nutritional and metabolic status; nutritional support; pancreatic sepsis; sepsis; acute pancreatitis, pancreonecrosis.

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

Introduction

The prognosis and progression of critical illness, including pancreatogenic sepsis, are significantly influenced by the life support systems that maintain homeostasis. It is essential to recognize that the pancreas, through its exocrine and endocrine functions, plays a critical role in maintaining the body homeostasis by participating in digestion and metabolism. Disruption of these processes can lead to the development of severe nutritional and metabolic deficiencies, resulting in the hypermetabolism/hypercatabolism syndrome. This syndrome is characterized by increased energy intake and nitrogen losses, as well as a significant decrease in total plasma protein levels with severe hypoalbuminemia [1, 2]. This syndrome is common in many critical conditions, but has specific features in severe acute pancreatitis. It is associated with the catabolic nature of the disease, the specificity of its pathogenesis, the need for extensive surgical intervention, and the rapid development of sepsis, which further increases catabolism and the body's energy requirements, worsening the course and prognosis of the disease [3, 4].

Intensive therapy for pancreatogenic sepsis should focus on supporting the vital functions of the body, which deteriorate due to the development of multiple organ failure syndrome. Special attention should be paid to nutritional deficiencies, which are often underestimated in the management of this condition.

Nutritional support (NS) is not only about providing nutrition to patients, but also includes a range of measures aimed at maintaining trophic

homeostasis, optimizing structural, functional and metabolic processes, and preserving adaptive reserves [5]. It can also be seen as a tool for managing the systemic inflammatory syndrome, reducing the number of complications, and modifying the course of the disease [6,7]. Insufficient NS in acute pancreatitis and other surgical abdominal diseases can have negative effects on the cellular and humoral components of immunity, leading to a decrease in the body's non-specific reactivity, slower healing processes, and the development and progression of enteral insufficiency syndrome, which is a crucial element in the pathogenesis of infected pancreatic necrosis [8,9].

The aim of this article was to identify patterns in the development of diagnostic techniques for nutritional and metabolic status and management of NS in patients with pancreatogenic sepsis.

Materials and Methods

To obtain a comprehensive overview of the current scientific knowledge, a thorough search was performed using 3 databases, namely PubMed, Scopus, and Elibrary, from 2018 to 2023. The keywords used for the search were acute pancreatitis, infected pancreatic necrosis, sepsis, nutritional support, metabolism, and indirect calorimetry. The search yielded 95 eligible articles, including 16 meta-analyses and 6 systematic reviews that met the inclusion criteria. The category restriction was set to randomized clinical trials and reviews in patient groups younger than 18 years and older than 60 years. The inclusion criteria for the review were based on design (clinical trials published in international peer-reviewed journals without language or national restrictions) and subjects (adult patients with pancreatic necrosis and sepsis). The authors extracted data from the selected articles, including the author's first and last name, journal name, country, and year of publication.

Results of the Study

The initial search yielded 486 articles, of which 52 were in Russian and 434 were in English. After excluding articles that did not meet the search criteria, 133 articles remained, from which clinical observations and articles that did not meet the inclusion criteria were again excluded. A total of 95 articles were included in the systematic review, comprising 16 meta-analyses and 6 eligible systematic reviews. The source selection algorithm is shown in the figure.

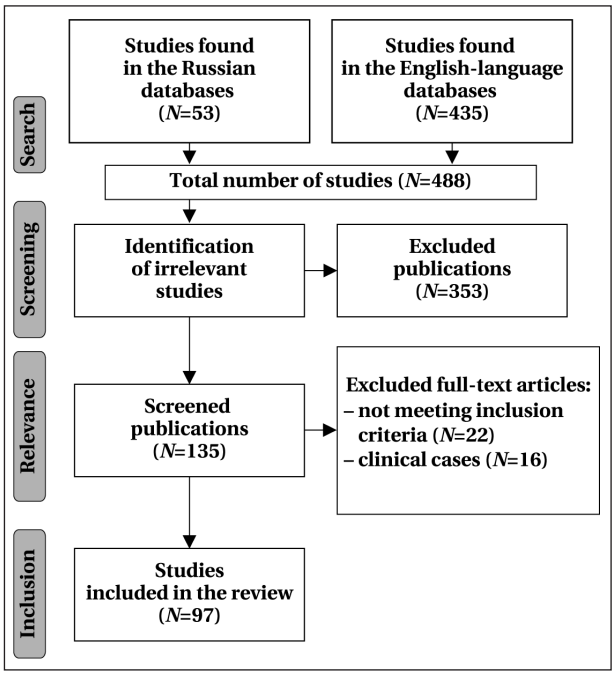

Fig. Flowchart of the source selection.

Indices and scales. Rational and timely monitoring of nutritional and metabolic status is an important aspect of intensive therapy of pancreatogenic sepsis in the ICU. To date, there are no uniform algorithms and protocols for monitoring the nutritional and metabolic status of patients with severe acute pancreatitis (AP). However, there are a large number of different indices and scales to assess the risk and severity of nutritional deficiency in critically ill patients, although no index or scale has shown its prognostic value in surgical patients in the ICU [10, 11]. In a study based on the analysis of the nutritional status of 120 critically ill patients, the NRS-2002 was shown to have the highest sensitivity and specificity among all scales for the detection of nutritional risk [12, 13]. In another study, it was found that the prognostic significance of such popular scales for assessing the severity of nutritional deficiency in surgical patients as NRS-2002, MUST, MNA-SF is still unclear, and the most appropriate scale, despite its lower specificity but comparable in strength of prognostic ability to APACHE-II and SOFA scales, is the mNUTRIC scale [14, 15]. However, to date, no studies have determined its prognostic value in patients with pancreatogenic sepsis. Large-scale studies are needed to determine appropriate scales to assess the severity of nutritional deficiencies in this patient population based on compliance with the requirements of practical medicine, including ease of use and interpretation of results, informativeness, and reliability and validity confirmed by studies conducted in clinical settings [16, 17].

Notably, ESPEN recommendations state that all critically ill patients (including those with severe AP) admitted to the ICU should initially be considered at high risk of malnutrition, which means that the use of prognostic indices to identify the risk of malnutrition is currently inappropriate [18].

Indirect calorimetry (metabolography). Indirect calorimetry is a valuable tool for studying nutritional and metabolic disorders in patients

suffering from various diseases. It is based on the determination of a patient's current energy requirements based on the simultaneous measurement of oxygen consumption (VO_2) and carbon dioxide elimination (VCO_2) during spontaneous breathing or lung ventilation [19]. In addition, this method allows real-time calculation of a patient's energy requirements and assessment of nutrient metabolic pathways, both of which are critical in planning nutritional and metabolic support for critically ill patients [20].

Patients with severe AP have higher resting energy requirements than healthy individuals because they develop septic complications and a marked hypermetabolism/hypercatabolism syndrome. The randomized TICACOS trial demonstrated improved survival with daily metabolographic monitoring of patient energy requirements and appropriate daily adjustment of NS composition [21, 22].

Indirect calorimetry can measure a patient's energy requirements much more accurately than calculated formulas, preventing both over- and undernutrition and identifying indications for supplemental parenteral nutrition or, conversely, avoiding unnecessary prescriptions [23, 24]. However, the use of this method is limited for a variety of reasons, including the high cost of the necessary equipment, insufficient training of physicians in clinical nutrition, and a lack of medical literature on the use of indirect calorimetry in critically ill patients [25]. Certain limitations in the use of indirect calorimetry in the ICU contribute to the continued use of outdated equations in clinical practice, the prognostic value of which is increasingly questioned [26].

Anthropometry. Anthropometry is a non-invasive and relatively straightforward research method that involves measuring the basic parameters of the human body and its components. Anthropometric methods include the measurement of height and weight, BMI, subcutaneous fat thickness, upper arm circumference, and other human parameters that provide the practitioner with some insight into the patient's condition. However, it remains unclear which of these parameters are the most informative and can be used to assess the severity of nutritional deficiency in patients with pancreatogenic sepsis [27]. For example, the commonly used body mass index may not be an effective indicator for assessing nutritional deficiency due to various factors such as fluid therapy, diuresis, and other fluid losses [28]. Therefore, large randomized trials are needed to determine the predictive and prognostic value of different anthropometric parameters in patients with nutritional deficiencies.

Intra-abdominal hypertension. Multiple organ failure developing in infected pancreatic necrosis can be caused by progression of septic complications as well as intra-abdominal hypertension resulting in abdominal compartment syndrome, which is a serious and potentially fatal complication in surgery and intensive care [29]. Destructive acute pancreatitis is a major cause of abdominal compartment syndrome [30, 31]. Other factors causing intra-abdominal hypertension include intestinal paresis, duodenal compression, gastric stasis, and the presence of free fluid in the abdominal cavity and retroperitoneum due to enzyme-containing effusion, abdominal wall stiffness due to edema, and inadequate analgesia [32]. It is important to note that the intestinal failure syndrome, which is a consequence of intestinal paresis in these patients, plays an important and sometimes critical role in the pathogenesis of intra-abdominal hypertension. Therefore, it is essential to develop an adequate approach to correct intestinal paresis in patients with severe AP complicated by sepsis. This approach is necessary to address several issues simultaneously, such as the progression of the intestinal failure syndrome, the development of intra-abdominal hypertension, continuous translocation of intestinal flora into the bloodstream, and ischemic injury to the intestinal mucosa with dystrophic changes in the epithelium, which can lead to dangerous complications such as intestinal perforation and peritonitis [33–35].

Intra-abdominal hypertension affects many systems of the body, including the cardiovascular, urinary, and respiratory systems, but the organs of the digestive tract are of particular interest because of their role in the pathological process and the development of changes prior to clinically detectable signs of abdominal compartment syndrome. Inadequate fluid therapy with underlying heart failure and renal dysfunction further exacerbates the process, and the emerging and rapidly progressing intestinal mucosal edema and paresis lead to a disruption of intestinal barrier function with continued translocation of intestinal flora into the abdominal cavity and systemic blood flow, closing the «vicious circle». This requires a radical change in the strategy of nutritional and metabolic support and control of intra-abdominal hypertension. Further large-scale studies are needed to identify the most effective methods to reduce the severity of intra-abdominal hypertension and to establish clear indications for switching from enteral to parenteral nutrition and vice versa in patients with pancreatogenic sepsis in the ICU. Patients with pancreatogenic sepsis and intra-abdominal hypertension are more likely to have increased energy requirements due to decreased intestinal perfusion, acidosis, or bacterial translocation.

Analgesia. Adequate analgesia is one of the most important components of intensive care for severe acute pancreatitis. A recent systematic review and meta-analysis aimed to compare the efficacy of different methods of analgesia in acute

pancreatitis [36]. Despite its infrequent use, epidural analgesia has been shown to be more effective than medication and should be considered as an alternative or as a component of combined analgesia when used with analgesics in a multimodal approach [37, 38].

Thoracic epidural anesthesia is particularly attractive from the point of view of nutritional and metabolic support and intensive care for several reasons.

First, the early use of prolonged epidural anesthesia in patients with severe AP, in addition to its analgesic effect, also has an enteroprotective effect, which is beneficial for the treatment of intestinal paresis and prevention of abdominal compartment syndrome, which in turn significantly affects the nutritional and metabolic support strategy [39].

Second, thoracic epidural anesthesia can block afferent stimuli that serve as triggers for the development of endocrine and metabolic responses to stress, indirectly reducing the intensity of catabolism.

Third, with adequate fluid therapy, thoracic epidural anesthesia improves splanchnic blood flow, thereby reducing the clinical manifestations of acute pancreatitis.

Fluid therapy. Organ and system injury in severe acute pancreatitis is primarily the result of intoxication and hypovolemia. Adequate fluid therapy is the only treatment for this disease that has been associated with a reduction in mortality in large studies over the past decade [40]. According to some authors, the blood supply to the pancreas can decrease by more than 70% immediately after the first manifestations of acute pancreatitis [41]. In addition, hypovolemia leads to hypoperfusion of all internal organs, resulting in progression of intestinal paresis and enteral failure syndrome with further impairment of intestinal barrier function, progression of infectious complications and multiple organ failure syndrome [42]. According to experts, an infusion started on the first day of the disease may prevent or reduce damage to the pancreas by maintaining a minimally adequate microcirculation [43]. In particular, adequate fluid therapy should precede nutritional support, which is ineffective in the presence of signs of dehydration in patients with severe AP.

Data regarding the fluid volume required for infected pancreatic necrosis are conflicting. The benefit of goal-directed fluid therapy in acute pancreatitis (reduction of heart rate below 120/min, achievement of mean arterial pressure of 65–85 mm Hg, restoration of diuresis to 0.5–1.0 mL/kg/h) remains unproven. Hematocrit, lactate, urea, and creatinine may be considered laboratory markers of volume status and adequate tissue perfusion; therefore, their serial measurement is recommended [44]. The determination of splanchnic blood flow in the pancreas can be used to assess the efficacy of fluid therapy, but studies on the use of pancreatic Doppler imaging as a prognostic marker of the severity of acute pancreatitis and as a method to assess the efficacy of treatment are extremely limited.

Enteral or Parenteral Nutrition? For a long time, parenteral nutrition was preferred in patients with severe AP, despite the high risk of catheter-associated infections, electrolyte disturbances, progression of multiple organ failure syndrome, and high cost of parenteral nutrition drugs [45–47]. The use of this type of NS allowed «pancreatic rest» and reduced the intensity of its exocrine secretion, thus minimizing the local inflammatory response caused by enzymatic aggression [48, 49].

New knowledge about the role of intestinal nutrition in the pathophysiology of acute pancreatitis has changed the approach to the principles of intensive therapy for this disease [50]. The results of meta-analyses conducted over the last decade, including a different number of randomized controlled trials with different numbers of participants, clearly showed the advantages of enteral nutrition over parenteral nutrition in terms of the incidence of complications (infectious and non-infectious), need for surgical intervention, progression of multiple organ failure syndrome, and mortality [51–53].

A 2018 meta-analysis of 5 RCTs (348 patients) showed that the use of enteral nutrition was associated with a significant reduction in mortality, RR 0.36 (95% CI: 0.20–0.65), and the incidence of organ dysfunction, RR 0.39 (95% CI: 0.21–0.73), compared with parenteral nutrition [52]. These differences were confirmed in a recent meta-analysis of 11 studies, involving 562 patients. The results showed that enteral nutrition significantly reduced mortality (RR=0.43; 95% CI: 0.23–0.78), risk of complications (RR=0.53; 95% CI: 0.39–0.71), and length of hospital stay (mean difference=–2.93, 95% CI: –4.52 to –1.34) [53].

The American Association of Clinical Nutrition and Metabolism ASPEN recommends the use of parenteral nutrition only when enteral nutrition is not possible or cannot meet the minimum caloric requirements of the body [54].

Specific indications and contraindications for the administration of enteral and parenteral nutrition should be considered when providing nutritional and metabolic support to patients with pancreatogenic sepsis.

Early enteral nutrition. The timing of NS initiation is a key point in the management of patients with AP, including those with infected pancreatic necrosis. The concept of «pancreatic rest» has been popular since the 1970s [55, 56]. This concept states that enteral nutrition should be initiated only after complete relief of abdominal pain and normalization of blood pancreatic enzyme levels. Based on the

concept of minimizing pancreatic stimulation, parenteral nutrition and its gradual expansion, starting with clear liquids, have been used. However, this concept is based only on speculation, has no reliable evidence base, and its implementation may lead to worsening of the patient's condition and increase the risk of developing an unfavorable outcome.

In contrast, the popularity of early enteral nutrition is «gaining momentum» worldwide, and not by chance [57]. The American Gastroenterological Association guidelines, published in 2013 and updated in 2018, recommend early (within the first 24 hours) enteral nutrition for acute pancreatitis [58–61]. This recommendation is supported by a meta-analysis of five large randomized controlled trials, the results of which clearly demonstrate the benefits of early enteral nutrition through positive effects on the structure and function of the intestinal epithelial layer, which inhibits the translocation of intestinal flora into the systemic bloodstream and internal organs [62,63].

A 2018 systematic review evaluating the results of 10 randomized controlled trials showed that in infected pancreatic necrosis, initiation of enteral nutrition within the first 48 hours resulted in less progression of systemic inflammatory response and multiple organ failure, need for surgical intervention, and mortality compared with delayed enteral or parenteral nutrition [64, 65].

Enteral formulas. Most studies on the clinical benefits of early enteral nutrition have used semi-elemental enteral formulas, while more recent studies have used standard polymeric formulas. All the studies demonstrated the feasibility of using both elemental enteral formulas in patients with pancreatitis.

In a small RCT of 30 patients, both formulas were found to be safe and well tolerated. Visual analog scale parameters and number of bowel movements per day were evaluated. Some clinical advantages of semi-elemental enteral formulas were found, including a shorter ICU stay (23±2 vs. 27±1 days, P=0.006) and no weight loss [66].

Another meta-analysis involving 428 patients showed no differences in the incidence of infection and mortality between patients receiving formulas with different elemental compositions [67].

A more recent meta-analysis of 15 RCTs (1376 participants) showed no benefit from any specific enteral formula [68].

Nevertheless, it is clear that patients with severe AP are at a high risk of malabsorption; therefore, semi-elemental enteral formulas may be of great interest. Given the wide variety of enteral formulas available in the market, further large randomized clinical trials are needed to identify the optimal enteral formulation for patients with pancreatogenic sepsis.

Routes of enteral nutrition administration. There are no definitive answers in the literature as to which method of enteral nutrition delivery is most effective, has a lower risk of complications such as induction of local inflammation, and is preferable for use at any given time during the course of the disease.

Based on a 2014 multicenter randomized trial in patients with pancreatitis, no advantage was found for enteral feeding via nasogastric tube in the first 24 hours of illness compared to oral feeding 72 hours after the onset of illness. This study included only 205 patients, which limited the power to detect a significant difference between the study groups. In addition, one-third of patients required enteral nutrition via nasogastric tube because of lung ventilation or intolerance to oral nutrition.

According to the scientific literature, the small intestine has long been the preferred site for tube placement. Enteral nutrition delivered to the GI tract proximal to the ligament of Treitz stimulates pancreatic enzyme secretion [69, 70]. Traditionally, this has been thought to lead to increased pancreatic autolysis and further progression of acute pancreatitis. There is experimental and clinical evidence that exocrine pancreatic secretion is not stimulated when enteral nutrition is administered into the duodenum distal to the ligament of Treitz. Such a route of administration can be easily accomplished in the current context by endoscopic methods or intraoperatively. In addition, several studies have shown that nasojejunal administration results in a significantly higher volume of absorbed nutrition than nasogastric administration [71]. This method of administration has advantages in patients with severe AP due to impaired gastric motility, with the degree of delayed gastric emptying increasing with disease severity.

The underlying mechanism of these abnormalities is primary gastric motility dysfunction with impaired proximal and distal gastric coordination as a result of hormonal imbalance. A recent meta-analysis comparing the efficacy of nasogastric and nasojejunal delivery of enteral nutrition in 131 patients found no differences in safety, efficacy, or mortality.

Another meta-analysis of 220 patients fed via nasogastric or nasointestinal tube also found no significant difference between groups in mortality, incidence of complications (infectious and non-infectious), diarrhea and need for surgery, severity of pain, food intolerance, and severity of protein-energy deficiency syndrome. A large multicenter trial, which was discontinued due to the inability to recruit participants, was designed to help select the preferred method of enteral nutrition administration [72].

It is believed that if prolonged (30 days or more) nasoenteral nutrition is required, alternative routes of administration should be considered, as

prolonged tube placement can lead to complications such as nasopharyngeal trauma, sinusitis, tube displacement and removal, «silent» aspiration, etc. [71]. Gastrostomy, jejunostomy, or gastrojejunostomy may be used as an alternative route for enzyme administration, but research on their efficacy in severe acute pancreatitis is limited.

Rate of nutrient delivery. It is important to note that the rationale for enteral NS in patients with severe AP depends on the rate of enteral formula delivery, the mode of administration (continuous infusion, cyclic, or bolus), and the initial volume of enteral nutrition.

Despite the paucity of scientific papers on this topic, current clinical guidelines recommend the use of continuous feeding because of its better tolerability [73]. They also state that patients in the ICU should not receive energy in amounts corresponding to metabolic needs determined by indirect calorimetry or calculated formulas. Based on previous studies [73], the risk of mortality in acute critical illness, including pancreatogenic sepsis, is minimized when 70–80% of the energy requirement measured by indirect calorimetry is provided.

In addition, a large study of the timing of initiation of parenteral nutrition in critically ill patients, including 4640 participants, showed that administration of significant amounts of energy during the first 24 hours in the intensive care unit was associated with an increase in complications. All patients received enteral nutrition. In addition, group 1 received parenteral nutrition from day 1 and group 2 from day 8 in the ICU. The authors found that late initiation of parenteral nutrition was associated with a decrease in infectious complications and ventilator days, and reduced the need for renal replacement therapy [74]. In other studies, patients with increased energy intake were more likely to have episodes of hyperglycemia requiring high doses of insulin [75, 76].

Parenteral nutrition. Despite the benefits of enteral nutrition, approximately 20% of ICU patients require parenteral nutrition, which is currently considered the only form of NS in patients with enteral intolerance, high fistula, and gastrointestinal bleeding [77, 78]. Complications of severe acute pancreatitis may also lead to conditions that preclude enteral NS, such as intestinal obstruction, abdominal hypertension, abdominal compartment syndrome, and intestinal ischemia. Indications for parenteral nutrition may also include enteral intolerance and failure.

Total parenteral nutrition preparations have gained popularity because they combine the advantages of all single-component parenteral nutrition products, containing all necessary substances in one package, and are characterized by high bioavailability, ease of nutrient dosing, and minimal gastrointestinal side effects with intravenous administration.

Omega-3 fatty acids, which are included in a number of parenteral nutrition formulations, have systemic anti-inflammatory effects and may reduce the manifestations of multiple organ failure syndrome and improve clinical outcomes in severe pancreatitis.

A meta-analysis of eight randomized controlled trials showed that parenteral administration of omega-3 fatty acids reduced infectious complications, intensive care unit (ICU) length of stay, and mortality [79, 80].

The administration of parenteral formulas for pancreatic necrosis has unique characteristics. Hypertriglyceridemia is a proven factor in the severity of acute pancreatitis [81]; therefore, lipid emulsions should be administered by infusion pumps and controlled according to changes in the lipid profile. Elevated triglycerides are a limitation for the administration of lipid emulsions, including propofol, which should also be considered during NS [82].

Information on the use of two-component parenteral formulas that do not contain lipid emulsions is limited.

Parenteral vitamins and amino acids (glutamine, etc.) are also used for balanced parenteral nutrition. Four meta-analyses have been published on the use of glutamine in patients with AP. A meta-analysis of ten RCTs involving 433 patients with severe AP showed a significant reduction in infectious complications and mortality in patients receiving glutamine-enriched nutrition [83].

Another meta-analysis of 12 RCTs (505 patients) also showed a significant reduction in infectious complications and mortality after glutamine supplementation in patients with severe AP [79].

Two recent meta-analyses showed the beneficial effects of glutamine administration in patients with AP with increased serum albumin levels, decreased serum C-reactive protein levels, and reduced infectious complications and mortality [80, 84].

Nevertheless, the risk of bias in the studies listed cannot be excluded for many reasons, such as

- small sample size in most of the studies;
- possible heterogeneity of patients with regard to disease severity;
- incomplete analysis of other factors that may influence the outcome.

Macro- and micronutrient requirements. Patients with pancreatogenic sepsis, as in other critical conditions, require sufficient protein, fat, and carbohydrate, as well as micro- and macronutrients, to support homeokinesis of their metabolism [85, 86].

Indirect calorimetry is the «gold standard» not only for calculating the number of calories required, but also for studying the metabolic pathways of essential nutrients, and provides the most accurate real-time assessment of the body's needs.

The limitations of this method force clinicians to calculate proteins, fats, and carbohydrates em-

pirically, and there is no consensus on the amount of essential nutrients required for patients with pancreatic sepsis. Most commonly, 1.2–1.5 g/kg protein/day, 3–6 g/kg/day carbohydrates, and up to 2 g/kg/day lipids are recommended [87].

Previously published clinical guidelines have suggested a significant increase in protein intake in several categories of ICU patients [88]. A detailed analysis of the main sources of these recommendations revealed serious inconsistencies and a lack of an apparent evidence base [89, 90].

Information on the use of vitamins and trace elements in the intensive care of patients with pancreatogenic sepsis is limited.

Refeeding Syndrome. When initiating nutritional and metabolic support, refeeding syndrome should be considered, as it is particularly relevant for surgical patients in the ICU. Refeeding syndrome is a life-threatening condition characterized by metabolic derangements resulting from the resumption of nutrition in patients after prolonged fasting [91, 92]. Any type of nutrition (oral, enteral, or parenteral) can serve as a provoking factor. In addition, the risk of refeeding syndrome in critically ill patients is due more to stress-induced catabolism than to prolonged fasting [93, 94]. Clinical manifestations of refeeding syndrome include acute organ failure (cardiac, hepatic, renal), cerebral and cardiogenic pulmonary edema, thrombocytopenia, DIC, polyneuropathy, and cardiac arrhythmias [95].

To date, the only diagnostic criterion for refeeding syndrome is hypophosphatemia. However, many other conditions can cause low blood phosphate levels in ICU patients, which means that the specificity and prognostic significance of hypophosphatemia in the diagnosis of refeeding syndrome is low [96]. In addition, based on the results of the search for suitable predictors and scales to identify groups of patients at high risk for the syndrome, none of the scales studied showed sufficient specificity and prognostic significance.

Refeeding syndrome is a serious concern for patients with severe AP, and rational NS reduces the risk of its development [97].

Conclusion

Based on the analysis of selected sources, we found that all existing scales for assessing the severity of nutritional deficiency in patients with pancreatogenic sepsis have a low prognostic value, and the mNUTRIC scale is the most appropriate.

The use of parameters to assess the risk of nutritional deficiency according to the ESPEN clinical guidelines is inappropriate for ICU patients.

Indirect calorimetry has been shown to be the preferred method for estimating energy requirements in patients with pancreatogenic sepsis compared to routine calculation formulas.

Such «routine» anthropometric values as body weight, etc. are not always informative and prognostically significant in patients with severe AP in the ICU.

Analgesia, fluid therapy, and diagnosis and management of intra-abdominal hypertension are integral parts of intensive care in patients with pancreatic sepsis and are components of adequate NS.

Early enteral nutrition is the preferred technique for NS, and the choice of tube placement and the mode and rate of nutrient delivery remain controversial. The optimal composition of enteral nutrition for patients with pancreatogenic sepsis has not been specified, which is implicitly confirmed by the variety of enteral formulas available on the market.

We defined the role of refeeding syndrome as a life-threatening condition that develops when NS is initiated.

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The Editors of «General Reanimatology» journal find it important to update our audience on the current approaches to ethical evaluation in genomic research, specifically focusing on gene diagnosis and gene therapy. This is especially relevant due to the increasing emphasis on personalized critical care medicine and the ongoing search for genetic markers that can predict the course and outcome of critical illness and its complications.

Ethical Expertise for Gene Diagnostics and Gene Therapy Clinical Studies

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Summary

Purpose of the study: to develop proposals for improving regulatory documentation on ethical expertise (EE) of gene diagnostics and gene therapy clinical studies.

Materials and methods. We used general philosophical research methods, including the formal-logical, historical method, comparative method, systematic approach and axiological method. We analyzed 10 international acts, including acts of «soft law», 4 documents adopted at the level of interstate integration entities, 7 domestic normative legal acts, and a number of various doctrinal sources on the topic under consideration. In addition, we analyzed the regulatory documents for ethical committees (EC) acivities, including those from a historical perspective.

Results. We formulated the concept of EE, defined the principles and main areas of EE, including the personalized medicine, and suggested the regulatory principles of EE operating.

Conclusion. To improve the regulation of EE, the legal status and requirements for the activities of independent ECs should fit the scope of EE, differentially related to the area of a trial (non-interventional trials, clinical trials with a drug treatment); at the national level, independent ECs conducting EE of drug treatmemnt clinical trials should be institutionalized into a single system; to improve the activities of independent ECs in the field of clinical testing, the development of a special normative regulation is required.

Key words: ethical expertise; gene diagnostics; gene therapy; ethics committee; critical conditions; personalized medicine; reanimatology; legal regulation

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

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