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SEPTIC AND CRITICAL CONDITIONS IN CHILDREN ADMITTED TO THE INTENSIVE CARE UNIT: CLINICAL PROFILE, EARLY DIAGNOSIS, AND PROGNOSIS

THESIS SUMMARY

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ABBREVIATIONS:

PICU - Pediatric Intensive Care Unit
ICU - intraventricular haemorrhage
IMV - invasive mechanical ventilation
IC - informed consent
NIV - noninvasive ventilation
CBC - complete blood count
SMA - spinal muscular atrophy
ESR - erythrocyte sedimentation rate
AUC - Area under curve
CRP - C-Reactive Protein
INR - International Normalized Ratio
IQR - interquartile range
MALDI-TOF-MS - MALDI-TOF-MS mass spectrometry
N/A - not available
NPV - negative predictive value
PCT - procalcitonin
PELOD-2 - Pediatric Logistic Organ Dysfunction-2
PPV - positive predictive value
PRISM III - Pediatric Risk of Mortality III/Pediatric Risk of Mortality Score
PSS - Phoenix Sepsis Score/Phoenix Sepsis Scale
ROC - working characteristic curve
SIRS - Systemic Inflammatory Response Syndrome
SOFA (Sequential [Sepsis-related] Organ Failure Assessment)
pSOFA - Pediatric Sequential Organ Failure Assessment

I. INTRODUCTION

Sepsis continues to be a leading cause of morbidity and mortality, especially in the pediatric population. Historical analysis of this condition reveals the dynamic evolution of its definition, from early descriptions of "putrefactive" processes in antiquity to the modern concept that emphasizes dysregulated immune response and multi-organ damage. Although the introduction of different criteria in the definitions of sepsis over the years (Sepsis-1, Sepsis-2, Sepsis-3) has led to better diagnosis in adults, uncertainties still exist in children due to differences in age-related physiology, lack of specific diagnostic markers and limitations in the unified criteria.

Early recognition of sepsis and an appropriate therapeutic response are crucial to achieve a more favourable prognosis. Existing scoring systems (PRISM III, PELOD-2, pSOFA) and the most recent Phoenix Sepsis Score, although useful, do not always adequately reflect the rapidly changing condition of pediatric patients. In parallel, high-tech microbiological diagnostic methods such as MALDI-TOF-MS show potential for more rapid identification of the infectious agent but remain inaccessible to many clinical units. Biochemical laboratory indices used in routine practice (CRP, procalcitonin) demonstrate some weaknesses - low specificity and limited analytical precision.

Novel markers such as presepsin (sCD14-ST) and soluble mannose receptor (sMR; sCD206) represent promising predictors for timely detection of septic process, but there is still a lack of sufficiently reliable mechanisms for their measurement and validation, especially in the pediatric age group.

In view of the critical need to optimize diagnostic and therapeutic strategies in children, this dissertation focuses on the systematic review of established and innovative approaches for the recognition and assessment of sepsis in early life. This topic not only reflects the current scientific interest in multidisciplinary management of the disease but also draws attention to the poorly filled gaps in clinical practice in our country, the addressing of which would contribute to improved survival and quality of life in children.

II. AIM AND OBJECTIVES

Aim

To investigate the clinical profile and outcome of children with septic and critical conditions in a pediatric intensive care unit and to evaluate approaches to their early diagnosis

Objectives

1. To investigate the etiological structure of diseases in children with septic and critical conditions admitted to the PICU.

2. To assess the condition of the patients on admission to the ward using scoring systems: PRISM III, pSOFA, PELOD-2, Phoenix Sepsis Score.

3. To compare the performance of PRISM III, PELOD-2, pSOFA and Phoenix Sepsis Score in predicting the risk of developing complications in children with septic and critical conditions and to determine the most appropriate scale in the study group.

4. To evaluate sMR and Presepsin as innovative biomarkers of inflammation in septic and critically ill children and compare them with those established in clinical and laboratory practice.

5. To identify the combinations of biomarkers studied with the highest discriminative potential in septic and critical patients.

III. MATERIALS AND METHODS

1. Patients, criteria and study design

In the period 1 June 2022 - 31 January 2024, a prospective study was conducted among 80 children aged 7 days to 18 years, selected according to predefined criteria and hospitalized in the First Clinic with PICU of University Hospital "St. Marina" – Varna. The patients were divided into three main groups:

1) *Septic*: children with systemic inflammatory response syndrome (SIRS) caused by confirmed or suspected infection, meeting ≥ 2 criteria, one of which was changes in body temperature or leukocyte count (Group I)

2) *Critical*: children with SIRS of non-infectious origin (Group II)

3) *Control group:* children with no history or clinical evidence of infectious syndrome or SIRS (Group III).

For the study, the terms Group I and septic patients, as Group II, critical and non-infectious SIRS are interchangeable. Patients who developed a complication and developed organ dysfunction, as well as those without complications and no evidence of organ dysfunction, were considered equivalent categories within the study.

Inclusion criteria:

- Hospitalization in the PICU according to established internal criteria
- Clinical evidence of severe sepsis and/or critical condition
- Temperature > 38 °C or < 36 °C
- Heart rate outside the reference range for age
- Respiratory rate outside reference limits for age
- Oxygen dependence
- With or without comorbidities
- Signed informed consent (IC) by parent/guardian/custodian.

Study exclusion criteria:

- Participation in a clinical trial of medication
- Severe combined trauma in children
- Elective surgical patients prior to surgical intervention
- Patients with oncohematologic diseases and other forms of immune incompetence
- Age less than 7 days (early neonatal period)
- Hospitalization in the ICU > 15 days
- Patients with a history of adolescent pregnancy

In categorizing septic patients, the definition validated during the 2005 International Consensus on Pediatric Sepsis was used.

Extended patient history data were collected to obtain all information about the patients' condition. The complete questionnaire was based on systematic analysis and tailored to the specific aims and hypotheses of the study. It was administered as a structured interview at the patient-parent encounter after signed informed consent (IC) by the parent/guardian/custodian.

Physical Examination: Clinical examination was performed using basic propaedeutic methods of examination. Assessment of general condition was performed within 24 hours of admission to the paediatric intensive care unit using the pSOFA (Matics et al., 2017), PRISM III (Pollack et al., 1996), PELOD-2 (Leteurtre et al., 2013) and Phoenix Sepsis Score (Sanchez-Pinto et al., 2024) scales, the latter assessed retrospectively. The scoring systems were not administered to patients in the control group.

2. Laboratory methods

Blood was drawn from all participants at admission and before initiation of antibiotic therapy for complete blood count (CBC), blood gas analysis, coagulation, biochemical parameters (including CRP and PCT) and haemoculture.

By centrifugation of 2 ml of venous blood at room temperature, the resulting serum was stored according to the manufacturer's requirements until a single-arm study of innovative biomarkers of inflammation (sMR, Presepsin) was performed at a later stage of the study.

Sysmex XN 1000 (Sysmex Corporation, Japan) and Siemens Advia 1800 (Siemens Healthineers) devices were used for hematological and biochemical analyses.

The following reference values were used in the study:

- **Leukocytes:** age-dependent according to Heklotz et al.
- **CRP** (Siemens - Wide range C-reactive Protein) - 0.00 - 5.00 mg/L, cut-off - 20 mg/L - possible bacterial infection.
- **PCT** (Maglumi PCT, Snibe) 0.00 - 0.05 ng/mL, cut-off - > 0.5 ng/mL - possible bacterial infection; > 2.0 ng/mL - high risk of sepsis.

CRP and procalcitonin cut-off values were adopted according to the manufacturer's data (for PCT) and compared with international recommendations in Europe and the USA.

A manual ELISA method was used to determine the values of innovative biomarkers of inflammation (sMR and presepsin). The values of presepsin were determined with a Human Presepsin Elisa kit (ELISA Genie, Ireland) providing a measurement range up to 2000 pg/ml and with a sensitivity (detection threshold) of 11.64 pg/ml. The sMR values were determined with the Human Soluble Mannose Receptor kit (Hycult Biotech, Netherlands), with a measurement range of 3.1 to 200 ng/ml and a detection threshold of 3.1 ng/ml.

Microbiological testing of biological samples:

a. evidence of micro-organisms in blood (haemoculture)

- **automated** system for instrumental incubation of haemocultures (BACTEC, BD, USA)

b. microbial identification

- by MALDI-TOF Sirius mass spectrometry (Bruker, Daltonics) and MALDI-TOF-MS Sepsityper (Bruker, Daltonics)
- by PCR (Polymerase Chane Reaction) method: for SARS-CoV-2 and *Mycobacterium tuberculosis.* SaCycler-96 RT PCR System (Sacace, Italy), GeneXpert®Instrument System (Cepheid, Sweden) and Xpert MTB/RIF Ultra (Cepheid, Sweden) and SARS-CoV-2 Real TM kits (Sacace, Italy) were used.

c. antimicrobial susceptibility testing

- by automated VITEK 2 Compact system (bioMérieux, Marcy l'Etoile, France)
- by the Buyer-Kirby disk diffusion method

3. Statistical methods

Statistical analyses were performed using IBM Statistical Package for the Social Sciences, version 24 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 10.4.0 (GraphPad Software, CA, USA) and Python v3.13.0 (Python Software Foundation, OR, USA) and MedCalc version 23.1.6 (MedCalc Software Ltd).

In the descriptive analysis of parametric quantitative data, mean, standard deviation (SD), and minimum and maximum values were calculated. Non-parametric quantitative data were represented by median and interquartile range (IQR). For qualitative data, frequency and percentage distribution were used. Verification of normality of the distribution was performed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Mann-Whitney test was applied to compare non-parametric data. Statistical significance was determined at a $p \leq$ value of 0.05. Evaluation of ROC-curves included calculation of AUC, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). According to established criteria, a value of 0.7 was considered acceptable discrimination, 0.8 good discrimination, and 0.9 excellent discrimination. The optimal cut-off values were determined by Youden's Index maximizing sensitivity and specificity. The optimal combination of biomarkers was evaluated by multiple logistic regression supplemented with ROC analysis. Calibration of predicted rates was verified by comparison of observed and predicted event accrual probabilities. This involved calculating predicted values from the model, grouping into decile categories, and analyzing the observed frequencies within each category. To quantify the calibration, the Hosmer-Lemeshow test is used, which compares the predicted and observed probabilities using a statistical goodness-of-fit test. A $p \geq 0.05$ was considered an acceptable result, with significant deviations from these values indicating problems in the calibration model. To improve the performance of the rates that did not calibrate against our population, recalibration with new data was performed.

The Cox proportional hazards model was used to analyze the relationship between independent variables and the risk of an event occurring among study patients. Statistical significance was assumed at $p<0.05$ obtained from Wald test. Exp(B) (hazard ratio) interprets relative risk, with a value >1 indicating increased risk, < 1 indicating decreased risk, and ≈1 indicating no significant effect.

Calculating a reference interval using the robust method (Horn & Pesce, 2005). The robust method may be a preferred alternative to the percentile method, especially when the sample size is less than 120.

Prior to the start of the study, approval was obtained from the Research Ethics Committee of the Medical University “Prof. Paraskev Stoyanov” – Varna (Protocol No. 115/31.03.2022). All participants received written information regarding the nature and objectives of the project, along with an informed consent form.

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IV. RESULTS

1. Etiological structure of diseases in children with septic and critical conditions admitted to the PICU

The main characteristics of the 53 patients studied are presented in **Table 1**. Children in the age group 13-48 months (30.2%) had the highest relative proportion. these, 35.9% were dehospitalized and one fatality was recorded (1.9%).

Table 1. Clinical characteristics of patients with septic and critical conditions

Variables	Total n (%)	Septic n (%)	Critical n (%)
Mean age (months ±SD)	44 ± 56	39 ± 54	63 ± 62
Age			
7 - 28 days	7 (13.2)	7 (16.7)	0 (0.0)
29 days - 12 months.	15 (28.3)	12 (28.6)	3 (27.3)
13 months - 48 moths.	16 (30.2)	14 (38.1)	2 (18.2)
49 months - 108 moths.	5 (9.4)	2 (7.1)	3 (27.3)
109 months - 216 moths.	10 (18.8)	7 (9.5)	3 (27.3)
Gender			
Men	25 (47.2)	21 (50.0)	4 (36.4)
Female	28 (52.8)	21 (50.0)	7 (63.6)
Ethnicity			
Bulgarian	34 (64.1)	25 (59.5)	9 (81.8)
Turkish	7 (13.2)	7 (16.7)	0 (0.0)
Roma	11 (20.8)	9 (21.4)	2 (18.2)
Other	1 (1.9)	1 (2.3)	0 (0.0)
Total	53 (100)	42 (79.2)	11 (20.8)
Average length of stay in the PICU (days)	11 (1-50)	11 (1-50)	11 (3-26)
Source of admission to PICU			
Emergency pediatric department	36 (67.9)	26 (61.9)	10 (90.9)
Transferred from another unit in the same hospital	3 (5.7)	3 (7.1)	0 (0.0)
Transferred from another hospital	14 (26.4)	13 (31.0)	1 (9.1)
Chronic underlying diseases	13 (24.5)	11 (26.2)	2 (18.2)
Long-term sequelae after intensive care	5 (9.4)	2 (4.8)	3 (27.3)
Antibiotic therapy in the last 7 days before PICU admission	17 (32.1)	16 (38.1)	1 (9.1)
Oxygen therapy during the stay in the PICU			
With nasal cannula	45 (85.0)	37 (80.1)	9 (81.8)

Non-invasive mechanical ventilation (NIV)	4 (7.5)	4 (9.5)	0 (0.0)
Invasive mechanical ventilation (IMV)	4 (7.5)	1 (2.4)	2 (18.2)
Febrility on admission to the PICU	37 (69.8)	35 (83.3)	2 (18.2)
Outcome of the disease			
Discharged	17 (32.1)	14 (33.3)	3 (27.3)
Discharged and referred to social care	2 (3.8)	2 (4.8)	0 (0.0)
Dransferred to another unit in the same hospital	30 (56.6)	23 (54.7)	7 (63.6)
Transferred to another hospital	3 (5.6)	2 (4.8)	1 (9.1)
Fatal outcome	1 (1.9)	1 (2.4)	0 (0.0)

The etiological structure of illness in septic and critically ill children is shown in **Table 2** and **Figure 1**.

Table 2. Disease pattern of 53 septic and critically ill children hospitalized in the PICU between June 2022 and January.

Diseases	n (%)	Microbiologically confirmed infections n (%)	Complications encountered		Fatal outcome
			Type	n (%)	n (%)
Diseases with infectious etiology					
Respiratory Tract Infections	**27 (50.9)**				
Upper respiratory tract infections	5 (9.4)				
COVID-19 infection	4 (7.5)	SARS CoV-2$_4$ (7.5)	no	-	-
Laryngotracheitis	1 (1.9)		febrile seizure$_1$	1 (1.9)	-
Lower respiratory tract infections	22 (41.5)				
Pneumonia, unspecified	14 (26.4)		ARDS$_1$, hydropneumothorax$_1$, pleural effusion with lung abscess$_2$, pleural effusion$_3$	7 (13.2)	1 (1.9)
Aspiration pneumonia	1 (1.9)		no		-
Pneumococcal pneumonia	2 (3.8)	*Streptococcus pneumoniae*$_2$ (3.8)	pyothorax$_2$	2 (3.8)	-
COVID-19-associated pneumonia	1 (1.9)	SARS CoV-2$_1$ (1.9)	no	-	-
Pulmonary tuberculosis	1 (1.9)	*Mycobacterium tuberculosis*$_1$ (1.9)	TB pleuritis	1 (1.9)	-
Bronchiolitis, unspecified	2 (3.8)		no	-	-
Bronchiolitis associated with COVID-19	1 (1.9)	SARS CoV-2$_1$ (1.9)	no	-	-
Urinary Tract Infections	**2 (3.8)**				
Tubulointerstitial nephritis	1 (1.9)	*E. coli*$_1$** (1.9)	urosepsis	1 (1.9)	-
Tubulointerstitial nephritis	1 (1.9)	*Klebsiella oxytoca*$_1$ (1.9)	urosepsis	1 (1.9)	-
Gastrointestinal Tract Infections	**6 (11.3)**				
Rotavirus gastroenteritis	1 (1.9)	*Rotavirus*$_1$ (1.9	HUS	1 (1.9)	-
Rotavirus gastroenteritis	1 (1.9)	*Rotavirus*$_1$ (1.9)	encephalitis	1 (1.9)	-
Salmonellosis	1 (1.9)	*Salmonella Group D*$_1$ (1.9)	cerebral edema	1 (1.9)	-

Clostridial colitis	1 (1.9)	*Clostridioides difficile*$_1$ (1.9)	no	-	-
Enterocolitis, unspecified	1 (1.9)		no	-	-
Enterocolitis*	1 (1.9)	*Pseudomonas aeruginosa*$_1$ (1.9)	no	-	-
Central Nervous System Infections	**5 (9.4)**				
Encephalitis	3 (5.6)		no	-	-
Viral meningitis	2 (3.8)		no	-	-
Skin and soft tissue infections	**2 (3.8)**				
Pyoderma*	1 (1.9)	*Staphylococcus aureus*$_1$ (1.9)	upper limb abscess	1 (1.9)	-
Omphalitis*	1 (1.9)	*Enterobacter cloacae*$_1$ (1.9)	no	-	-
Total patients with diseases of infectious etiology	**42 (79.2)**	**18 (42.8)**		**17 (32.1)**	
Diseases of non-infectious etiology					
Nephrotic syndrome	1 (1.9)	n/a	anasarca	1 (1.9)	-
Cerebral artery thrombosis	1 (1.9)	n/a	cerebral infarction	1 (1.9)	-
Hemorrhagic disease of the newborn	1 (1.9)	n/a	intracerebral haemorrhage	1 (1.9)	-
Diabetes mellitus with ketoacidosis	2 (3.8)	n/a	ketoacidosis	2 (3.8)	-
Potassium permanganate poisoning	1 (1.9)	n/a	esophageal stenosis	1 (1.9)	-
Kawasaki disease	1 (1.9)	n/a	no	-	-
Hypotensive agent poisoning	1 (1.9)	n/a	no	-	-
Methadone poisoning	1 (1.9)	n/a	no	-	-
Status epilepticus, unspecified	1 (1.9)	n/a	no	-	-
Antidepressant poisoning	1 (1.9)	n/a	no	-	-
Total patients with non-infectious diseases	**11 (20.8)**	n/a		**6 (11.3)**	**0 (0.0)**
Total patients with infectious and non-infectious diseases	**53 (100)**	**-**		**23 (43.4)**	**1 (1.9)**

*HUS - haemolytic uraemic syndrome, TB - tuberculosis, ARDS - acute respiratory distress syndrome, $KnMnO_4$ - potassium permanganate, n/a - not applicable; *refers to neonate, **isolate has been proven in haemoculture and uroculture*

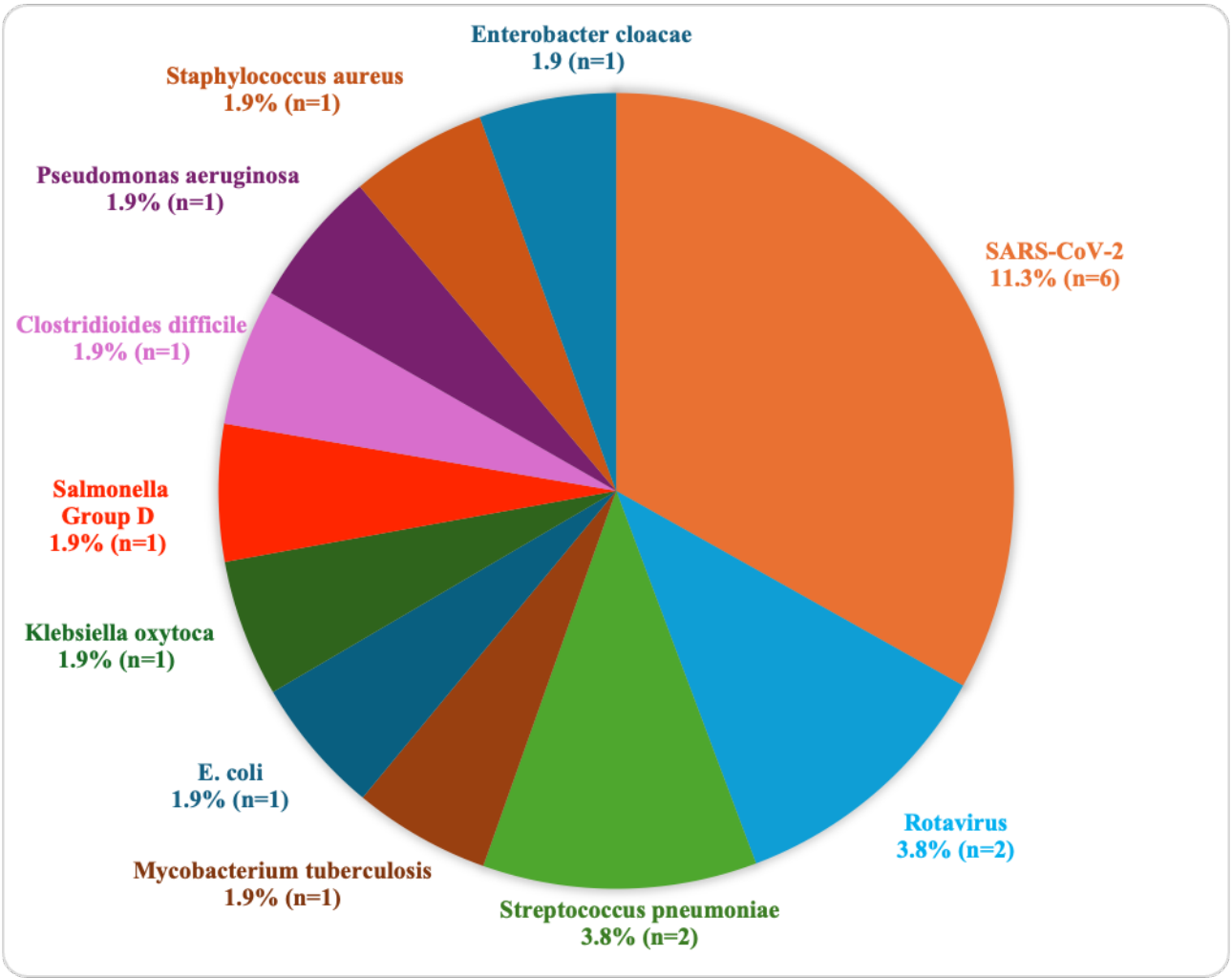


Figure 1. Etiological spectrum of microbiologically confirmed infections

The proportion of children with illnesses of infectious etiology was 79.2% (n=42), with microbiologically confirmed cases accounting for 42.8% (18/42). SARS CoV-2 and Rotavirus were responsible for 44.4% of illnesses with a proven microbial causative agent (8/18) (**Table 2**, **Figure 1**). Laboratory-confirmed bloodstream infection caused by *E. coli* (primary source urinary tract) was demonstrated in 1 septic patient.

In the group of children with SIRS of noninfectious origin, all hemocultures were negative.

The relative proportion of cases of noninfectious origin was 20.8% (11/53). Differences between septic and critical patients pertaining to key demographic and clinical variables are shown in **Table 1**.

Long-term sequelae after intensive care were found in 9.4% of those hospitalized **(Table 1)**.

Overall, 41.5% (22/53) of cases were diagnosed with lower respiratory tract infections, with microbiologically confirmed causative agents *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* and SARS-CoV-2. Infections of the gastrointestinal system were the second most common, with a proportion of 11.3% (6/53), with Rotavirus, *Salmonella Group D* and *Clostridioides difficile* among the identified pathogens.

Among children with SIRS of non-infectious origin, intoxications (n=4) and diseases related to endocrine and metabolic disorders, such as insulin-dependent diabetes mellitus with initial diabetic ketoacidosis (n=2), accounted for the largest relative proportion. The need for mechanical ventilation was reported in 15% (n=8) of those hospitalized. Underlying chronic diseases were recorded in 24.5% (n=13) of patients.

There were 23 deteriorations in general condition (43.4%) in the study groups, including one fatality (1/53). The distribution of secondary pathological conditions is presented in **Table 3**.

Respiratory and neurological complications were among the most common in the study cohort of patients, 18.8% and 9.4%, respectively. Pleural effusion was the most common respiratory complication, seen in three septic patients (5.6%). Neurological complications were recorded in 3.8% (n=2) of the SIRS group with no identified infectious agent.

2. Scoring systems

The clinical characteristics and rates studied in patients with/without complications are summarized in **Table 4**. Patients with complications had significantly longer ICU stays. Among the clinical scales used, only the PSS showed a statistically significant difference between the two groups (p=0.0023), with higher median values of all rates reported in patients with complications.

The prognostic reliability of the scales for predicting the risk of developing complications is presented in **Table 5**. PRISM III demonstrated similar accuracy (47.8%) in correctly predicting patients with PELOD-2 complications (43.5%). The pSOFA showed high reliability in patients without complications (88.0%), while the PSS best predicted septic patients who developed complications during the course of hospitalization (70.6%). PSS exhibited the highest overall predictive accuracy (76.2%) and good accuracy in both groups studied.

Table 3. Distribution of complications in septic and critical patients

Diagnosis[§]	All n (%)	Septic n (%)	Non-infectious SIRS n (%)
Respiratory system	***10 (18.8)***	***10 (18.8)***	***0 (0.0)***
ARDS[†]	1 (1.9)	1 (1.9)	0 (0.0)
Hydropneumothorax	1 (1.9)	1 (1.9)	0 (0.0)
Pleural effusion with lung abscess	2 (3.8)	2 (3.8)	0 (0.0)
Pleural effusion	3 (5.6)	3 (5.6)	0 (0.0)
Pyotorax	2 (3.8)	2 (3.8)	0 (0.0)
TB pleuritis	1 (1.9)	1 (1.9)	0 (0.0)
Nervous system	***5 (9.4)***	**3 (5.6)**	***2 (3.8)***
Febrile seizure	1 (1.9)	1 (1.9)	0 (0.0)
Cerebral edema	1 (1.9)	1 (1.9)	0 (0.0)
Encephalitis	1 (1.9)	1 (1.9)	0 (0.0)
Cerebral infarction	1 (1.9)	0 (0.0)	1 (1.9)
Intracerebral haemorrhage*	1 (1.9)	0 (0.0)	1 (1.9)
Other	***8 (15.2)***	***4 (7.6)***	***4 (7.6)***
Urosepsis	2 (3.8)	2 (3.8)	0 (0.0)
HUS	1 (1.9)	1 (1.9)	0 (0.0)
Soft tissue abscess of upper limb*	1 (1.9)	1 (1.9)	0 (0.0)
Anasarca**	1 (1.9)	0 (0.0)	1 (1.9)
Diabetic ketoacidosis	2 (3.8)	0 (0.0)	2 (3.8)
Esophageal stenosis	1 (1.9)	0 (0.0)	1 (1.9)
Patients with organ dysfunction	***23 (43.4)***	***17 (32.1)***	***6 (11.3)***
Patients without organ dysfunction	***30 (56.6)***	***25 (47.2)***	***5 (9.4)***
Total patients	**53 (100)**	**42 (79.3)**	**11 (20.7)**

*[§] - interpreted as complications in the course of the disease, ARDS - acute respiratory distress syndrome; † - complication resulting in death, TB - tuberculosis, HUS - haemolytic uraemic syndrome, *refers to a newborn, **patient with nephrotic syndrome*

Table 4. Analysis of clinical characteristics and scales in patients with/without complications

Variables	Group with OD n (%)	Group without OD n (%)	p
Mean age (months) ± SD	53±51	36±59	0.2603
Gender (male/female)	12/11	13/17	0.5320
Underlying diseases	4 (17.4)	9 (30.0)	0.2994
Febrility	16 (69.6)	21 (70.0)	0.9730
Mechanical ventilation	5 (21.7)	3 (10.0)	0.2450
Stay in PICU (days) ± SD	14 (±10)	8 (±5)	**0.0024**
Total	**23 (43.4)**	**30 (56.6)**	**<0.0001**

Scoring systems	median, (IQR)	median, (IQR)	p
PRISM III	6 (2-19)	2 (2-7.750)	0.1442
PELOD-2	3 (1-8)	2 (1-5)	0.3387
pSOFA*	5 (3-9)	4 (3-6)	0.2627
PSS*	2 (1-2)	1 (1-1)	**0.0023**

Mann-Whitney test, $p<0.05$

OD - organ dysfunction, SD - standard deviation, IQR - interquartile range, PRISM III - Pediatric Risk of Mortality III, PELOD 2 - Pediatric Logistic Organ Dysfunction 2, pSOFA - Pediatric Sequential Organ Failure Assessment, PSS - Phoenix Sepsis Score

**calculated by septic group*

Table 5. Prognostic reliability of PRISM III, PELOD-2, pSOFA and Phoenix Sepsis Score

Predicted patients	Observed patients without OD n (%)	OD n (%)	Total	OPA %
PRISM III*				
without OD n (%)	22 (73.3) [TN]	8 (26.7) [FN]	30 (56.6)	
with OD n (%)	12 (52.2) [FP]	11 (47.8) [TP]	23 (43.4)	63.3%
Total	34 (64.1)	19 (35.9)	53 (100)	
PELOD-2*				
without OD n (%)	21 (70.0) [TN]	9 (30.0) [FN]	30 (56.6)	
with OD n (%)	13 (56.5) [FP]	10 (43.5) [TP]	23 (43.4)	58.5%
Total	34 (64.1)	19 (35.9)	53 (100)	
pSOFA**				
without OD n (%)	22 (88.0) [TN]	3 (12.0) [FN]	25 (59.5)	
with OD n (%)	11 (64.7) [FP]	6 (35.3) [TP]	17 (40.5)	66.7%
Total	33 (78.6)	9 (21.4)	42 (100)	
PSS**				
without OD n (%)	20 (80.0) [TN]	5 (20.0) [FN]	25 (59.5)	
with OD n (%)	5 (29.4) [FP]	12 (70.6) [TP]	17 (40.5)	76.2%
Total	25 (59.5)	17 (40.5)	42 (100)	

*OD - organ dysfunction; OPA - overall prognostic accuracy; TN - true negative; FN - false negative; FP - false positive; TP - true positive; PSS - Phoenix Sepsis Score; *percentage calculated from the total number of patients (n=53); **calculated from the sepsis group (n=42).*

The discrimination and calibration of the four prognostic scales is presented in **Table 6**. Among the four models analyzed, PSS had the highest sensitivity (80.0%), NPV (80.0%), and discriminability (AUC 0.736, CI (0.576-0.897). The pSOFA, with 66.7% PPV, revealed the highest specificity (88.0%), PRISM III demonstrated moderate stratification (AUC 0.650), while PELOD-2 showed lower sensitivity (43.5%) and discriminatory ability (AUC 0.591) in the groups studied. The significance of calibration in the Phoenix Sepsis Score (Hosmer-Lemeshow test, Table 6 - χ^2=5.752, p=0.016) necessitated model improvement by recalibration. Graphical representations of the above methods are reflected in **Figure 2** to **Figure 6.**

Analysis with the Cox proportional hazards model found no statistically significant effect of any of the variables considered on time to event (**Table 7**). The best-found effect, although not significant, was related to the presence of underlying chronic disease of the patients (Exp(B)=1.718). Mechanical ventilation tended to reduce risk (Exp(B)=0.509), but without statistical significance.

Table 6: Discrimination and calibration of four prognostic scales

Metrics	PRISM III	PELOD-2	pSOFA*	Phoenix Sepsis Score*
Sensitivity %	47.8	43.5	35.3	**80.0**
Specificity %	73.3	70.0	**88.0**	70.6
PPV %	57.9	52.6	66.7	70.6
NPV %	64.7	61.8	66.7	80.0
AUC (CI)	0.650 (0.499-0.801)	0.591 (0.432-0.749	0.587 (0.401-0.773)	0.736 (0.576-0.897)
Hosmer-Lemeshow test, $\chi 2$	0.472 (p=0.790)	2.308 (p=0.511)	4.068 (p=0.254)	5.752 (p=0.016)

*PPV - positive predictive value, NPV - negative predictive value, AUC - area under the curve, CI - confidence interval, *studied for septic group only*
Hosmer-Lemeshow test, $\chi 2$ - chi-square, $p > 0.05$

Figure 2. Calibration for PRISM

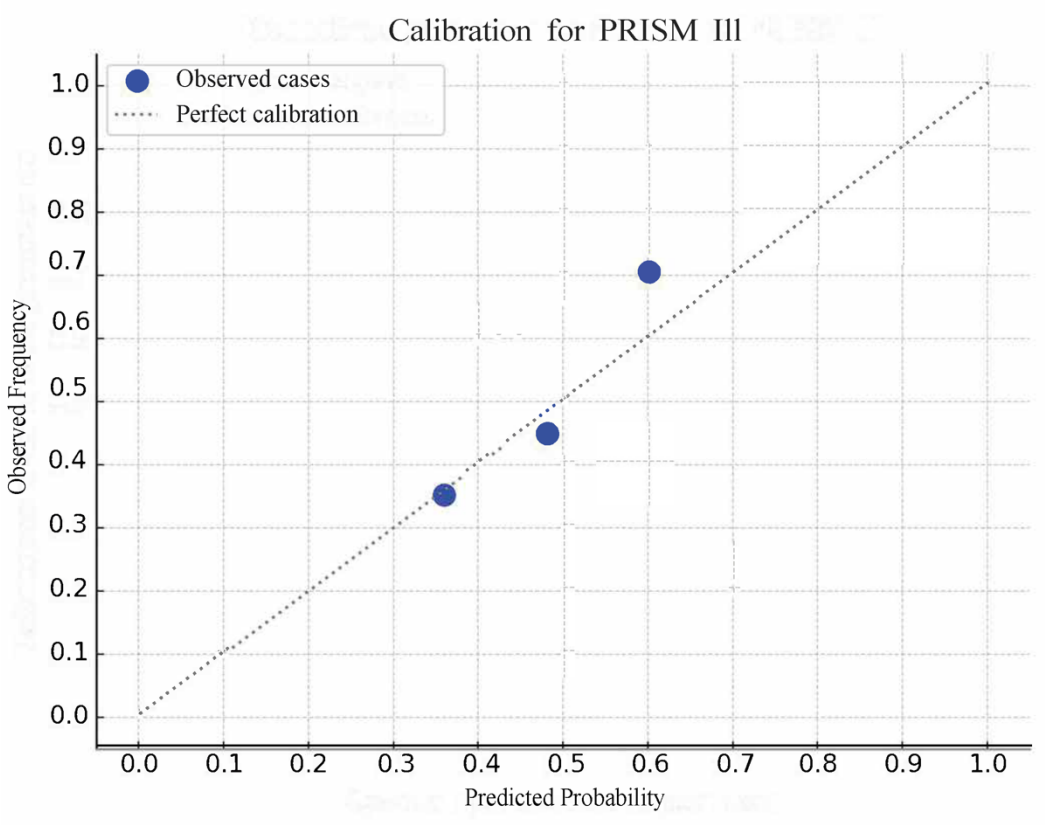


Figure 3. Calibration for PELOD-2

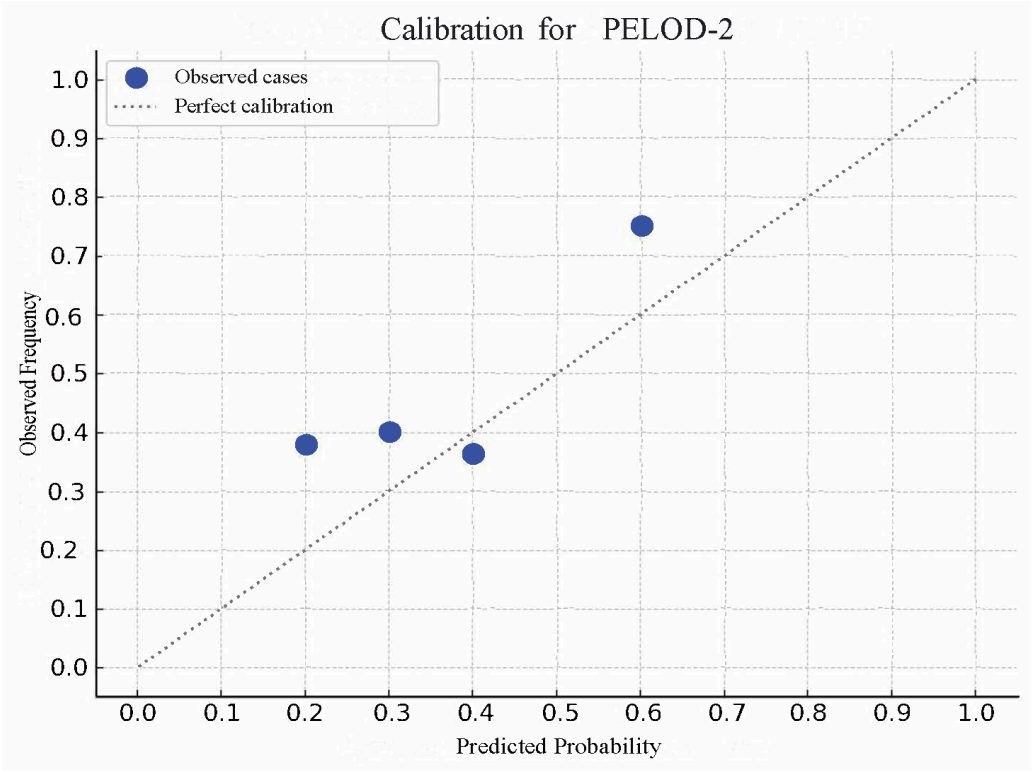


Figure 4. Calibration for pSOFA

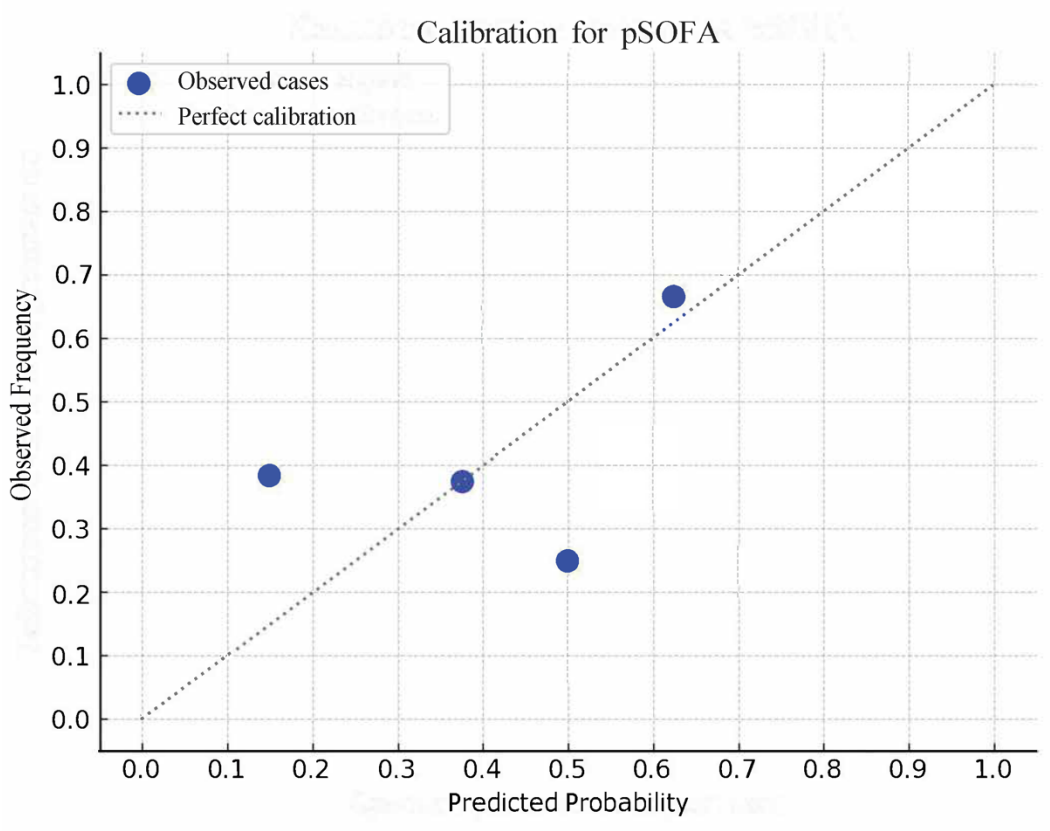


Figure 5. Calibration for Phoenix Sepsis Score

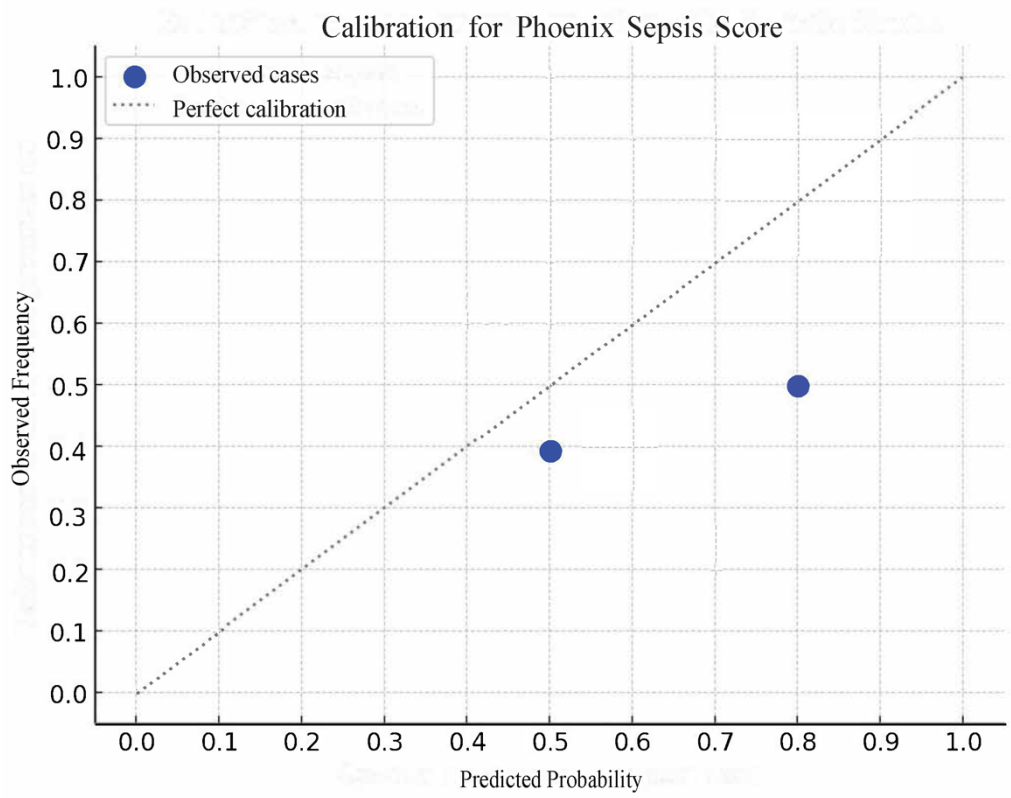


Figure 6. Recalibration for Phoenix Sepsis Score

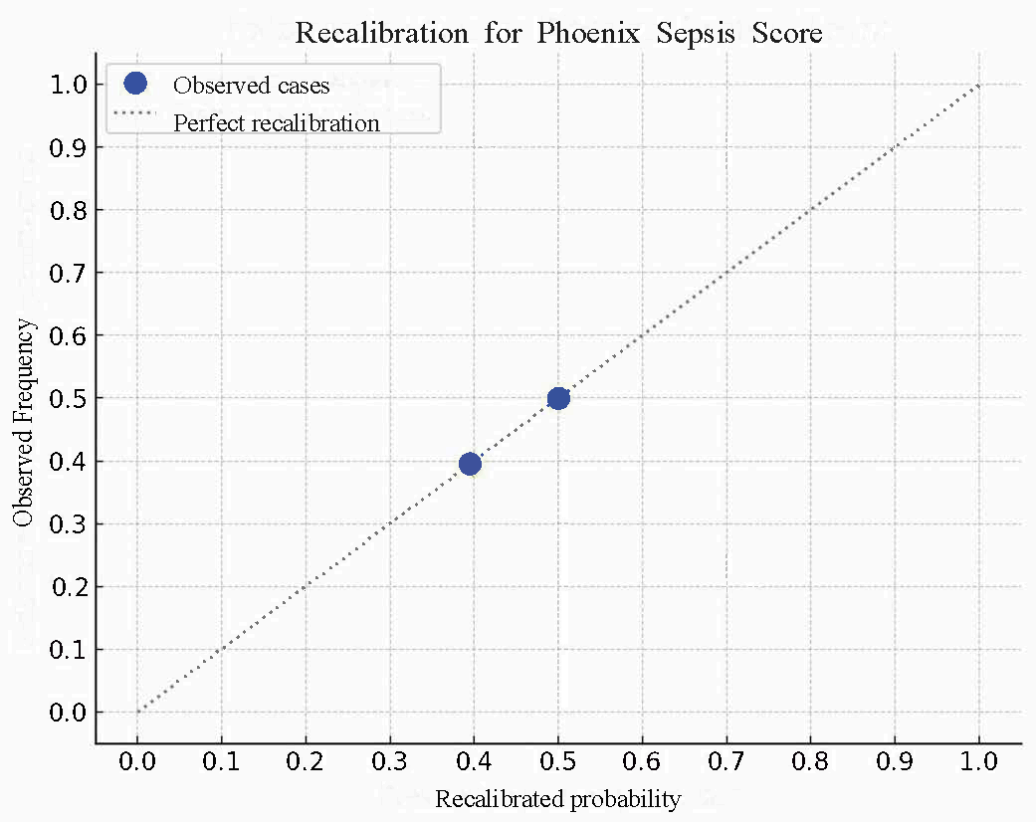


Table 7. Cox regression

Variable	B	SE	Wald	df	p	Exp(B)
Age	0.005	0.005	0.997	1	0.318	1.005
Gender	-0.264	0.502	0.276	1	0.599	0.768
Underlying chronic disease	0.541	0.605	0.801	1	0.371	1.718
Mechanical ventilation	-0.676	0.601	1.263	1	0.261	0.509

B - coefficient, SE - standard error, Wald - Wald test, df - degrees of freedom, p-value, Exp(B) - risk ratio

3. Laboratory biomarkers of inflammation

Table 8 presents the results of the laboratory parameters tested in the three groups. The median values of the five investigated inflammatory markers in the two patient groups (septic and non-infectious SIRS) showed a significant increase outside the indicated reference ranges. When compared with the control group, there was a statistically significant difference in all parameters between the two patient groups. The most pronounced significance was reported in septic patients (Group I, $p < 0.0001$).

In a direct comparison between the two groups of children with pathological conditions, the median CRP and procalcitonin values in sepsis (Group I) were found to be approximately twice as high as those in non-infectious SIRS patients. Despite the reported difference, statistical significance was not demonstrated. In contrast to CRP and procalcitonin, leukocyte, presepsin and sMR levels were similar in both groups, with no significant differences ($p = 0.4387$, $p > 0.9999$, $p = 0.7547$, respectively).

Boxplot analysis revealed that septic patients had the highest CRP levels compared to the other groups. The concentrations of presepsin in SIRS with a non-infectious causative agent were significantly elevated, with median values markedly higher compared to the other groups studied. The graphical representation of the laboratory parameters is visualized in **Figure 7**, next to **Figure 11**.

Reference values of presepsin (107.47 pg/mL) and sMR (152.81 ng/mL, 95% reference interval, right sided) were calculated by a robust statistical method based on the data from the control group of patients.

Table 8: Values of laboratory biomarkers in septic, critical patients and controls

Laboratory biomarker	**Group I** median (IQR)	**Group II** median (IQR)	**Group III** median (IQR)	**p (I vs. II)**	**p (II vs. III)**	**p (I vs. III)**
Leukocytes (10^9/L)	12.79 (8.60-17.41)	12.29 (11.02-23.85)	6.66 (5.67-8.12)	0.4387	**< 0.0001**	**< 0.0001**
CRP (mg/L)	17.20 (1.43-101.80)	6.73 (0.12-21.00)	0.60 (0.12-0.60)	0.1515	**0.0111**	**< 0.0001**
Procalcitonin (ng/mL)	1.57 (0.38-5.78)	0.95 (0.43-1.67)	0.05 (0.04-0.06)	0.1344	**< 0.0001**	**< 0.0001**
Presepsin (pg/mL)	228.10 (145.30-351.70)	347.00 (59.43-1136.00)	15.97 (3.52-51.10)	> 0.9999	**0.0009**	**< 0.0001**
sMR (ng/mL)	231.90 (191.20-307.20)	238.80 (138.90-349.60)	117.70 (106.50-125.60)	0.7547	**0.0024**	**< 0.0001**

Mann-Whitney test, $p \leq 0.05$
Group I, septic; Group II, noninfectious SIRS; Group III, controls; IQR, interquartile range.
Reference values - Leukocytes - age-dependent according to Heklotz et al., CRP - 0 - 5.0 mg/L, procalcitonin - 0.00 - 0.05 ng/mL, Presepsin - 107.47 pg/mL, sMR - 152.81 ng/mL (95% reference interval, right-sided)

Figure 7. Boxplot analysis of Leucocytes

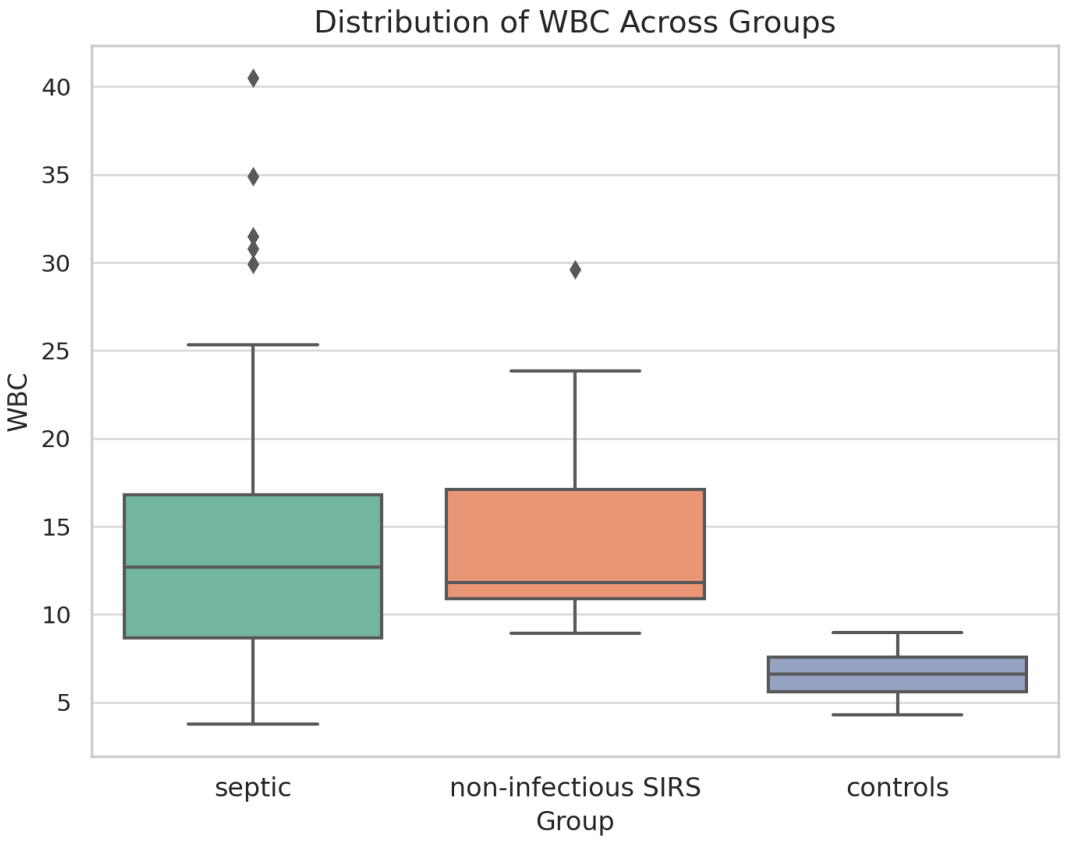


Figure 8. Boxplot analysis of CRP

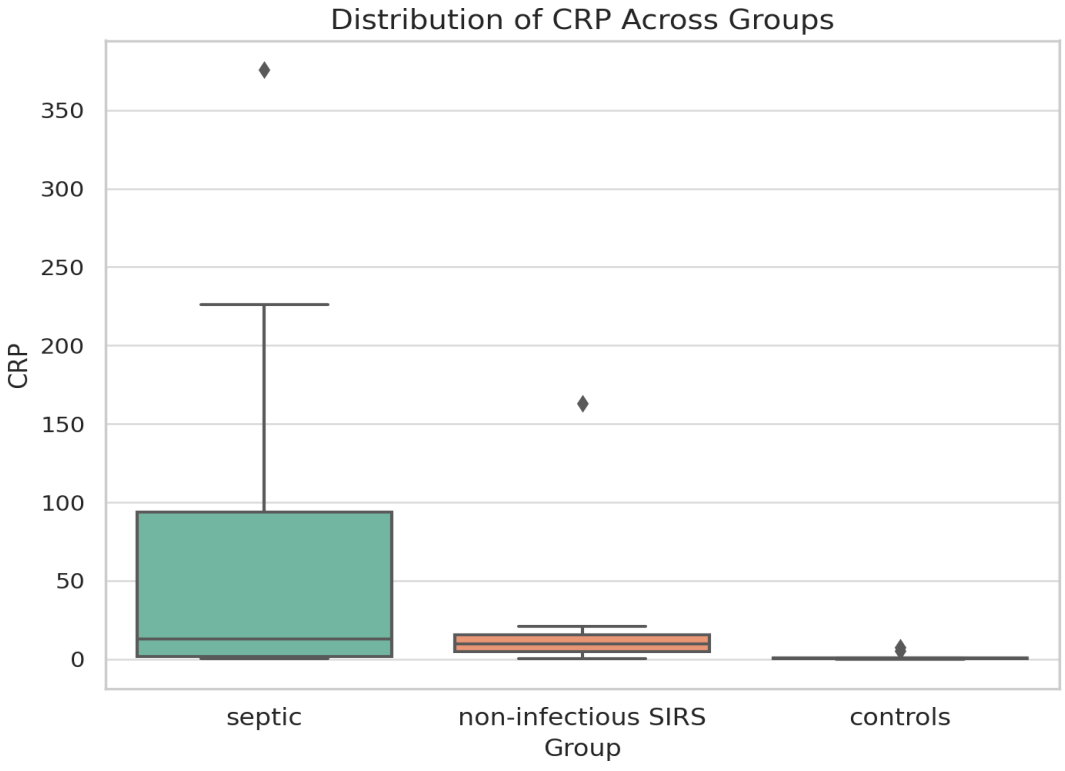


Figure 9. Boxplot analysis of Procalcitonin

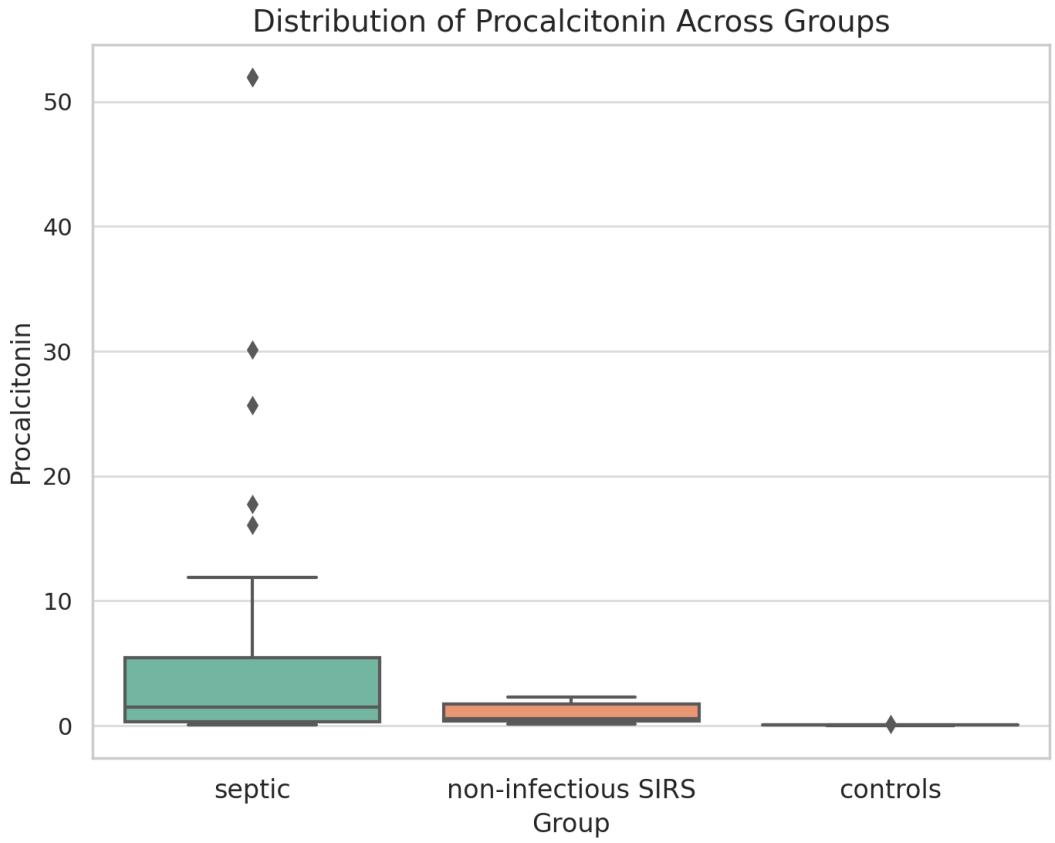


Figure 10. Boxplot analysis of Presepsin

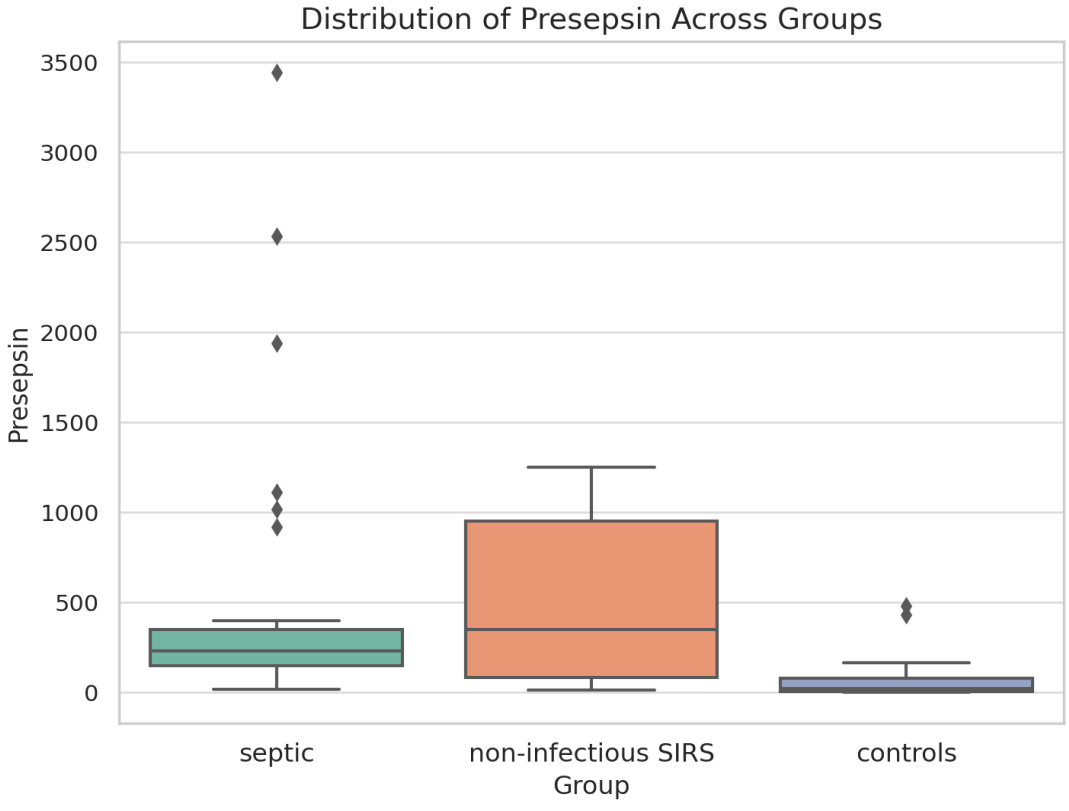


Figure 11. Boxplot analysis of sMR

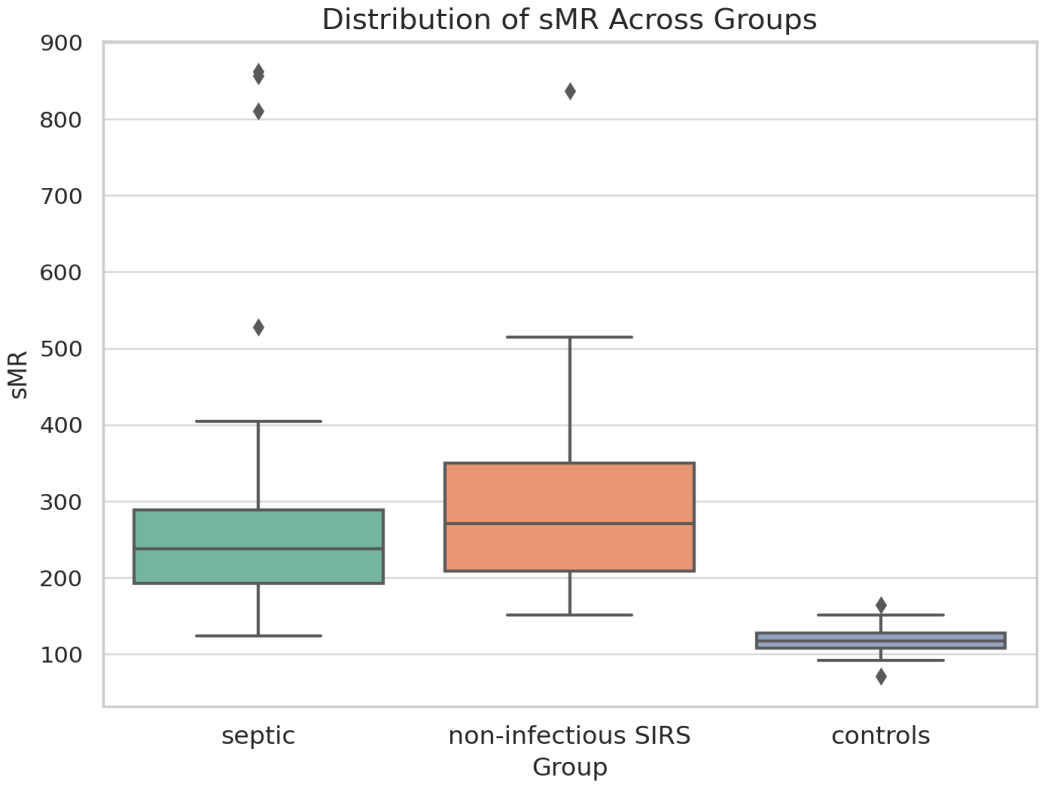


Table 10 presents an analogous analysis of performance in critical patients and controls For Procalcitonin (AUC = 1.0, 95% CI: 1.00-1.00) and leukocytes (AUC = 0.996, 95% CI: 1.00-1.00), extremely high diagnostic performance was reported. CRP demonstrated the lowest value (AUC = 0.761, 95% CI: 0.58-0.94). Lower sensitivity was shown by sMR (80%), presepsin (75%) and CRP (73%). All biomarkers had high specificity (≥ 88%), with sMR (96%), procalcitonin (96%) and leukocytes (93%) recording the lowest false-positive rates. The highest PPV was reported for procalcitonin (100%) and sMR (89%). Graphical representations of the ROC analyses of biomarkers of critical patients and controls are visualized in **Figure 17** to **Figure 21**.

Table 9. Diagnostic Accuracy and Reliability of Biomarkers in Septic Patients and Controls

Parameter	**Leukocytes (10^9/L)**	**CRP (mg/L)**	**Procalcitonin (ng/mL)**	**Presepsin (pg/mL)**	**sMR (ng/mL)**
AUC 95% CI	0.862 (0.58 - 0.88)	0.869 (0.64 - 0.93)	0.970 (0.86 - 1.00)	0.924 (0.69 - 0.95)	0.974 (0.76 - 0.99)
Cut-off	9.70	0.92	0.10	129.50	169.22
Sensitivity %	73	78	94	81	87
Specificity %	100	90	100	95	100
PPV %	100	93	100	96	100
NPV %	68	72	91	76	83
p value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

AUC - area under the curve, CI - confidence interval, PPV - positive predictive value, NPV - negative predictive value, Cut-off - cut-off point
Reference values - Leukocytes - age-matched, CRP - 0 - 5.0 mg/L, procalcitonin - 0.00 - 0.05 ng/mL,
Presepsin - 107.47 pg/mL, sMR - 152.81 ng/mL (95% reference interval, right-sided)

Figure 12. ROC analysis of leukocytes in septic patients and controls

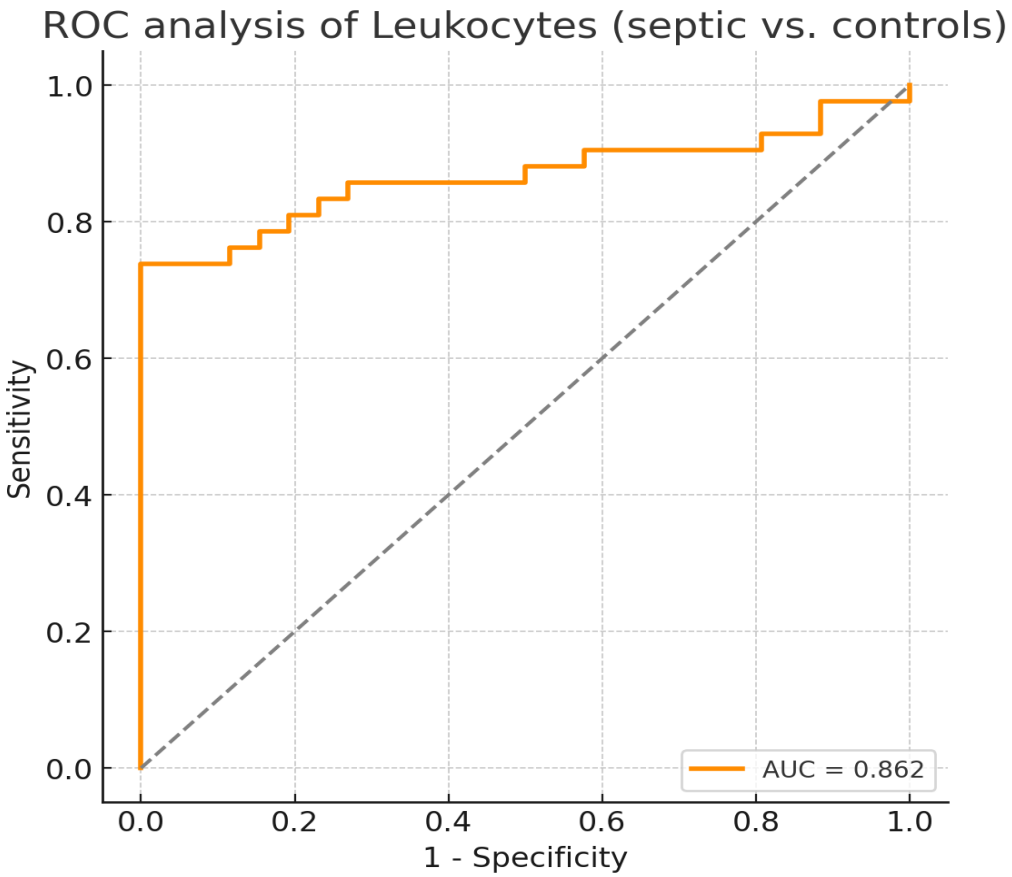


Figure 13. ROC analysis of CRP in septic patients and controls

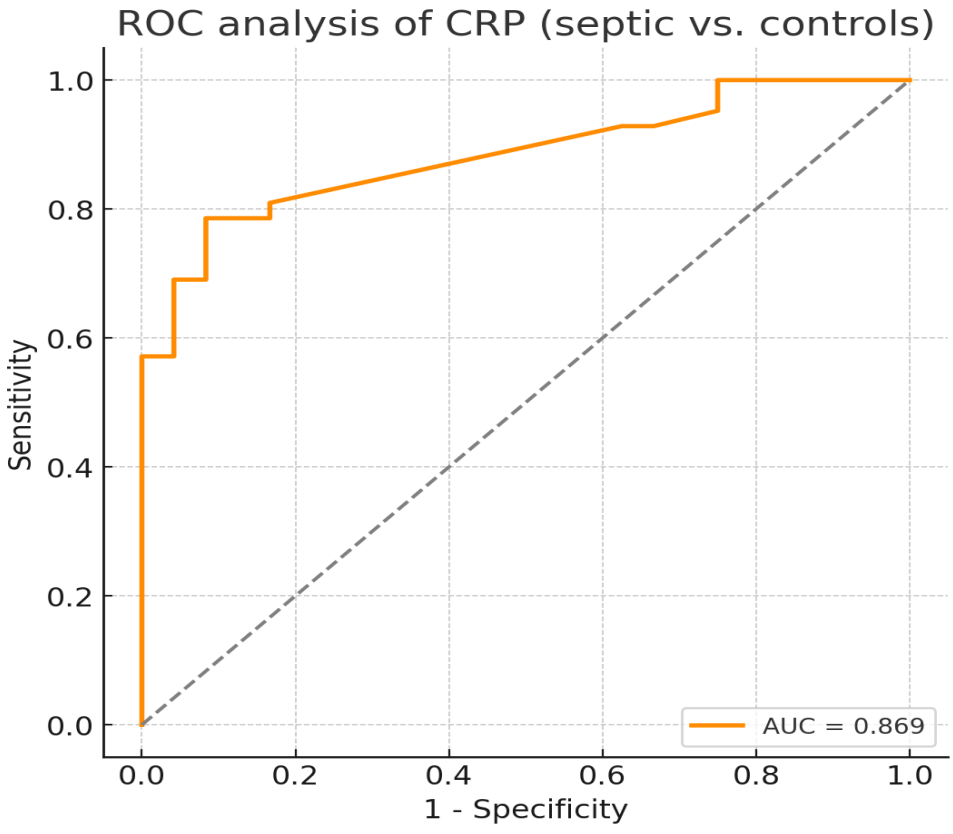


Figure 14. ROC analysis of Procalcitonin in septic patients and controls

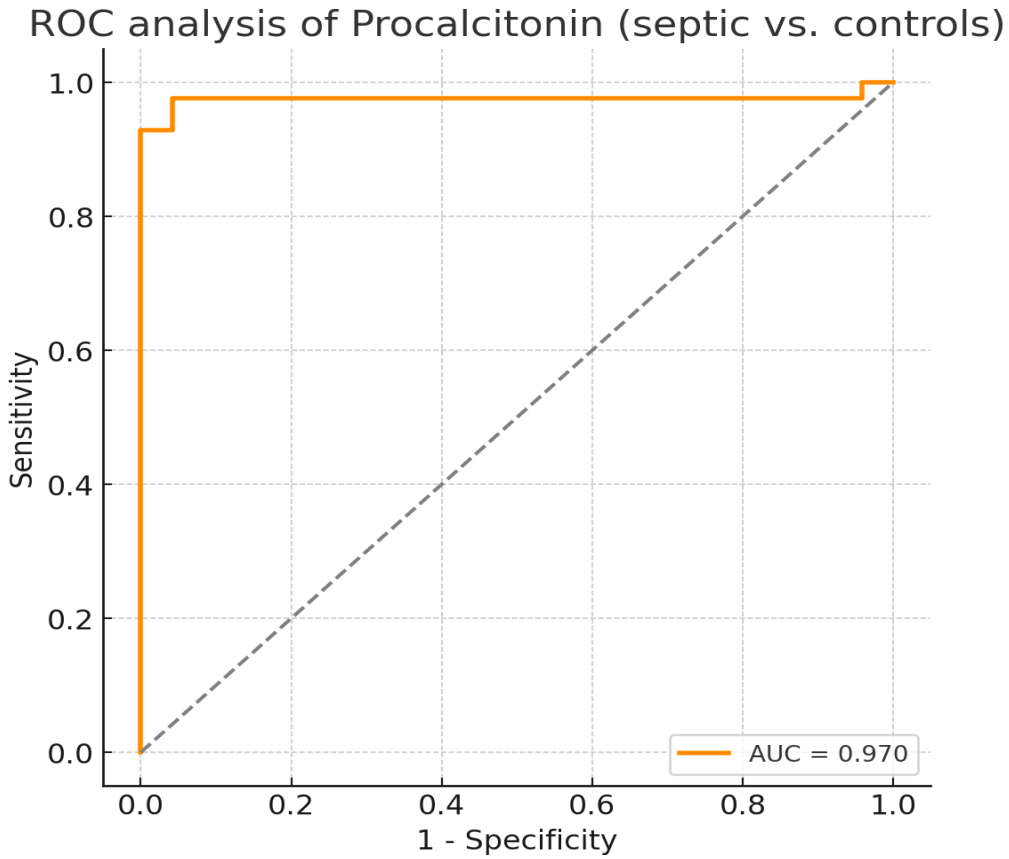


Figure 15. ROC analysis of Presepsin in septic patients and controls

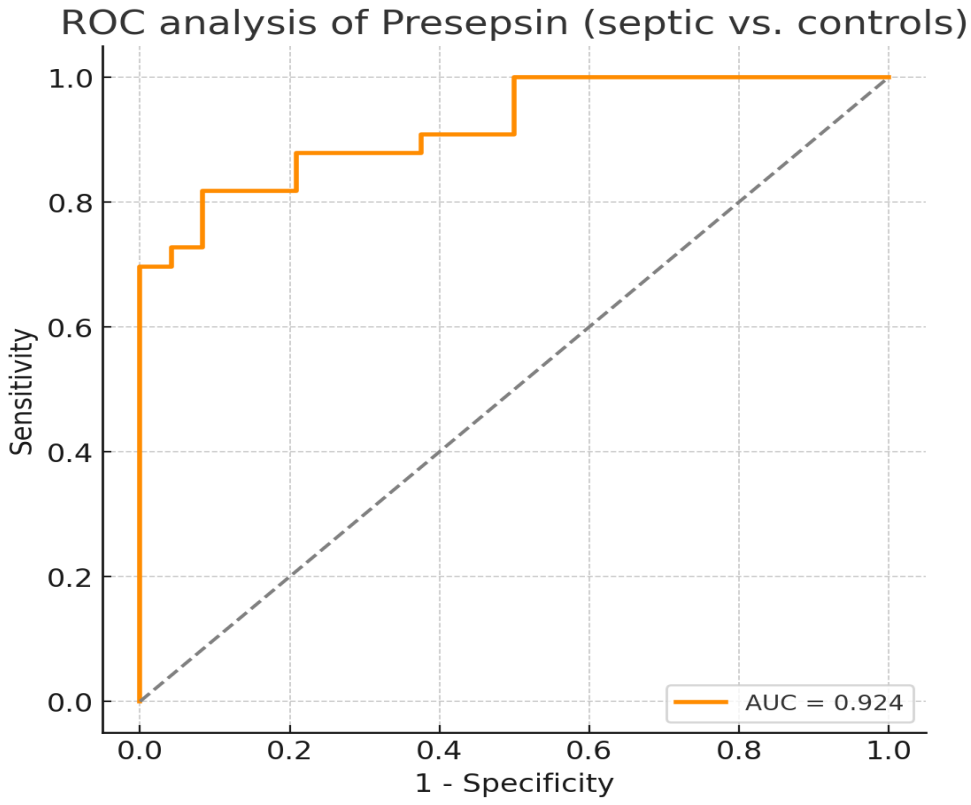


Figure 16. sMR ROC analysis in septic patients and controls

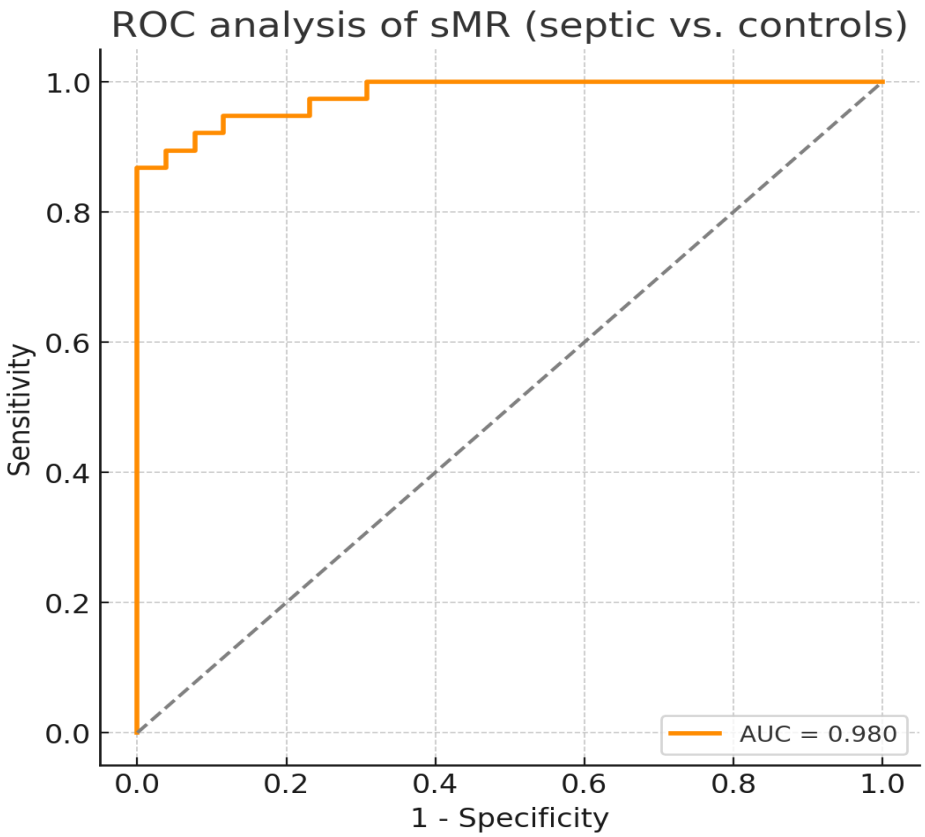


Table 10 presents an analogous analysis of performance in critical patients and controls For Procalcitonin (AUC = 1.0, 95% CI: 1.00-1.00) and leukocytes (AUC = 0.996, 95% CI: 1.00-1.00), extremely high diagnostic performance was reported. CRP demonstrated the lowest value (AUC = 0.761, 95% CI: 0.58-0.94). Lower sensitivity was shown by sMR (80%), presepsin (75%) and CRP (73%). All biomarkers had high specificity (≥ 88%), with sMR (96%), procalcitonin (96%) and leukocytes (93%) recording the lowest false-positive rates. The highest PPV was reported for procalcitonin (100%) and sMR (89%). Graphical representations of the ROC analyses of biomarkers of critical patients and controls are visualized in **Figure 17 to Figure 21**.

Table 10. Diagnostic performance and reliability of biomarkers in critical patients and controls

Indicator	**Leukocytes (10^9/L)**	**CRP (mg/L)**	**Procalcitonin (ng/mL)**	**Presepsin (pg/mL)**	**sMR (ng/mL)**
AUC 95% CI	0.996 (1.00 - 1.00)	0.761 (0.58 - 0.94)	1.0 (1.00 - 1.00)	0.875 (0.66 - 1.00)	0.819 (0.65 - 0.99)
Cut-off	8.92	0.80	0.12	92.20	151.56
Sensitivity %	100	73	100	75	80
Specificity %	93	92	96	88	96
PPV %	85	80	100	67	89
NPV %	100	88	92	91	93
p	**<0.0001**	**0.0111**	**<0.0001**	**0.0009**	**0.0024**

AUC - area under the curve, CI - confidence interval, PPV - positive predictive value, NPV - negative predictive value, Cut-off - cut-off point
Reference values - Leukocytes - age-matched, CRP - 0 - 5.0 mg/L, procalcitonin - 0.00 - 0.05 ng/mL,
Presepsin - 107.47 pg/mL, sMR - 152.81 ng/mL (95% reference interval, right-sided)

Figure 17. ROC analysis of Leukocytes in critical patients and controls

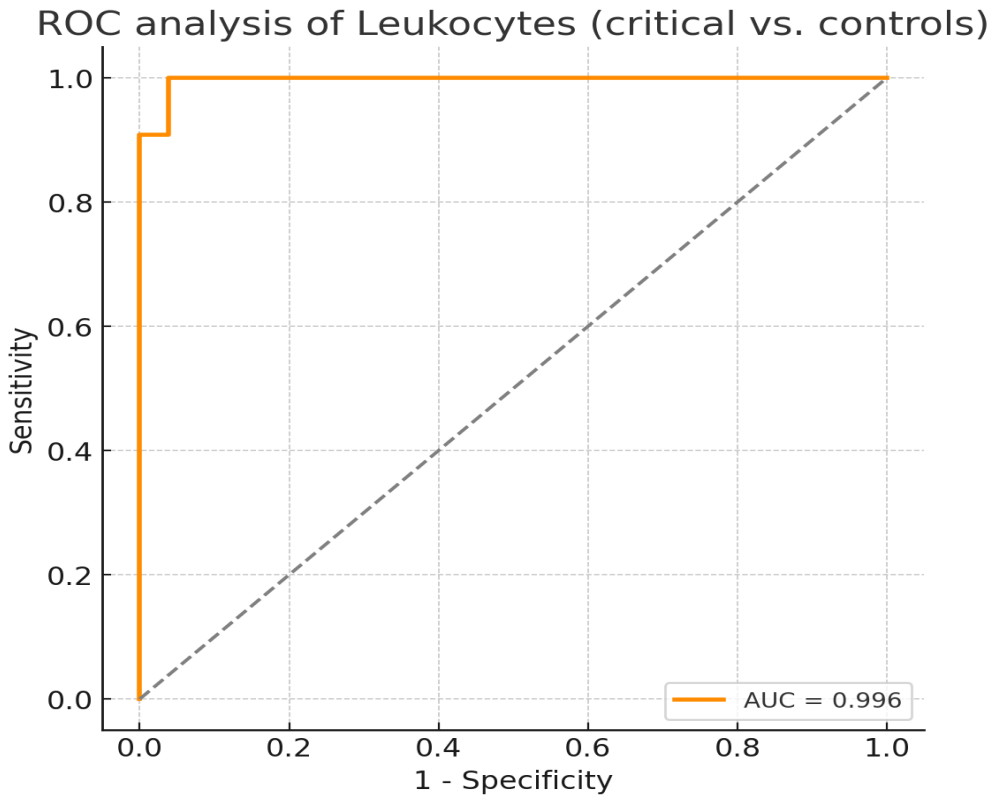


Figure 18. ROC analysis of CRP in critical patients and controls

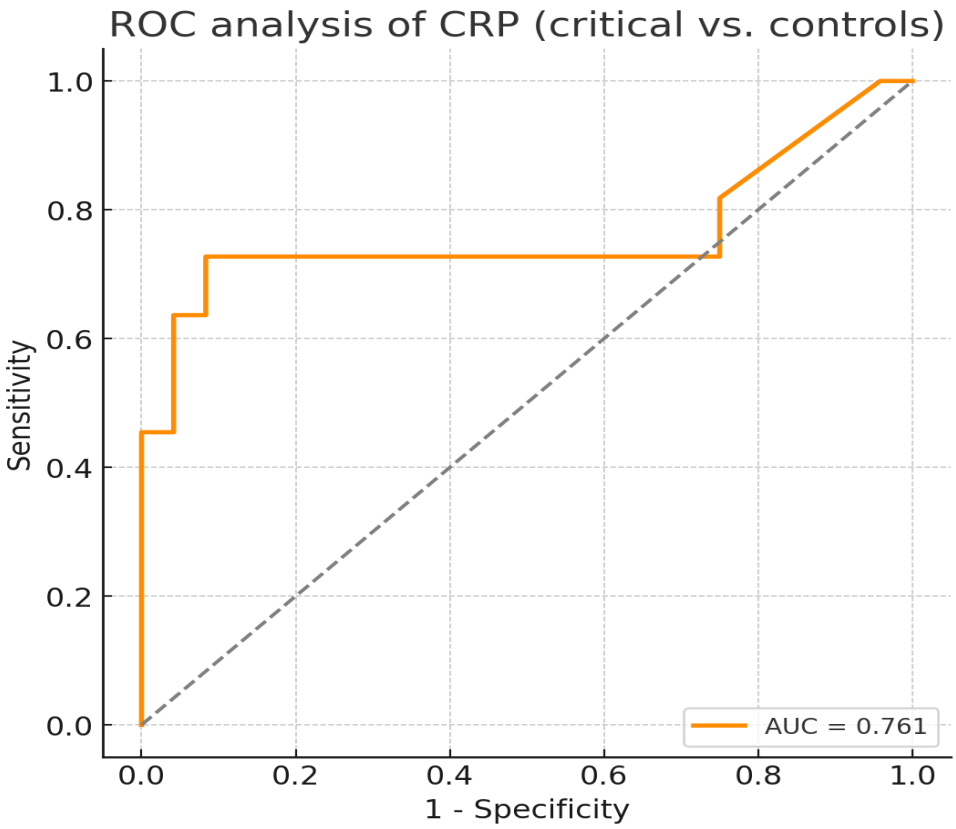


Figure 19. ROC analysis of Procalcitonin in critical patients and controls

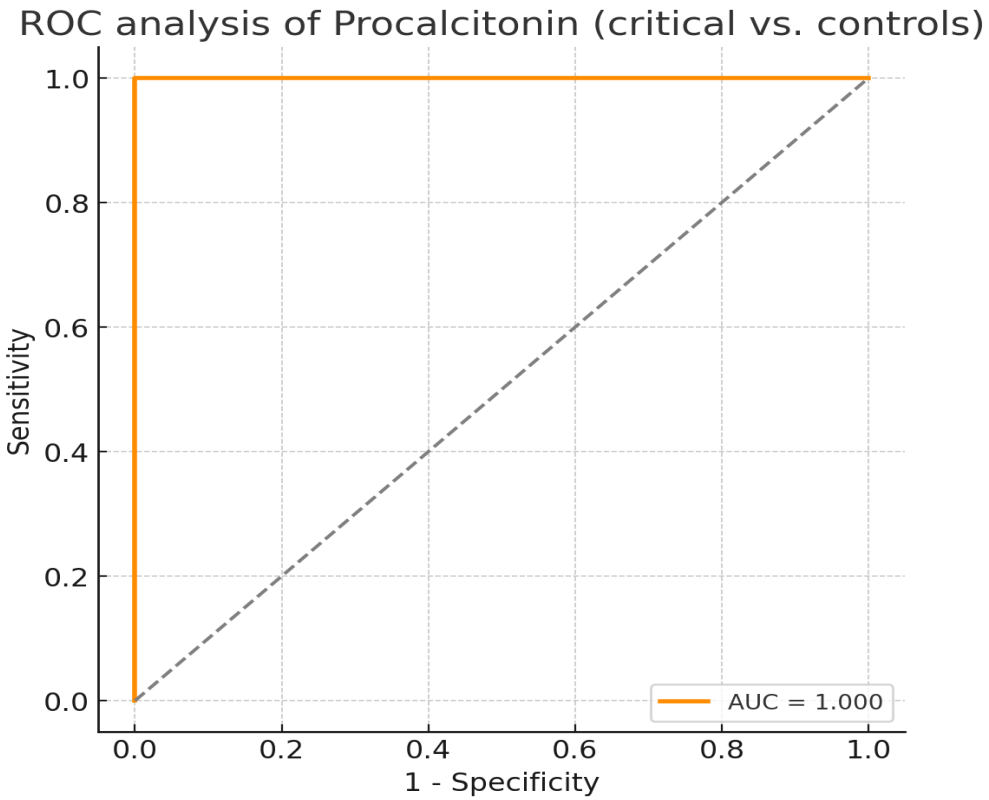


Figure 20. ROC analysis of Presepsin in critical patients and controls

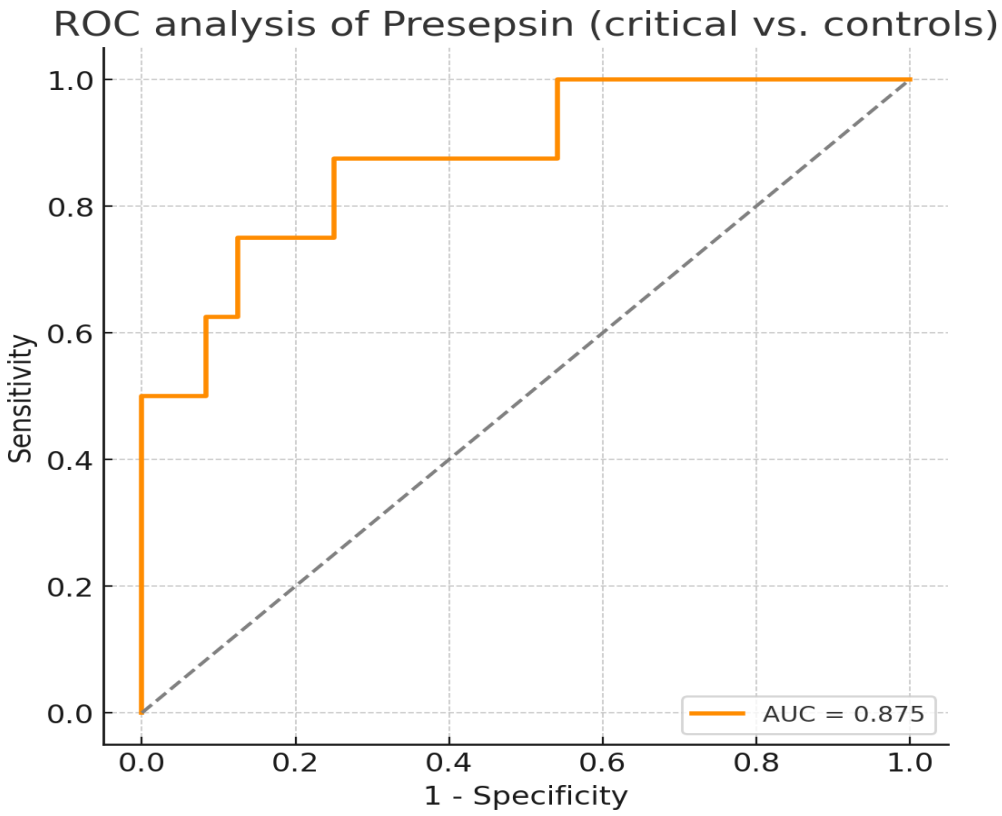
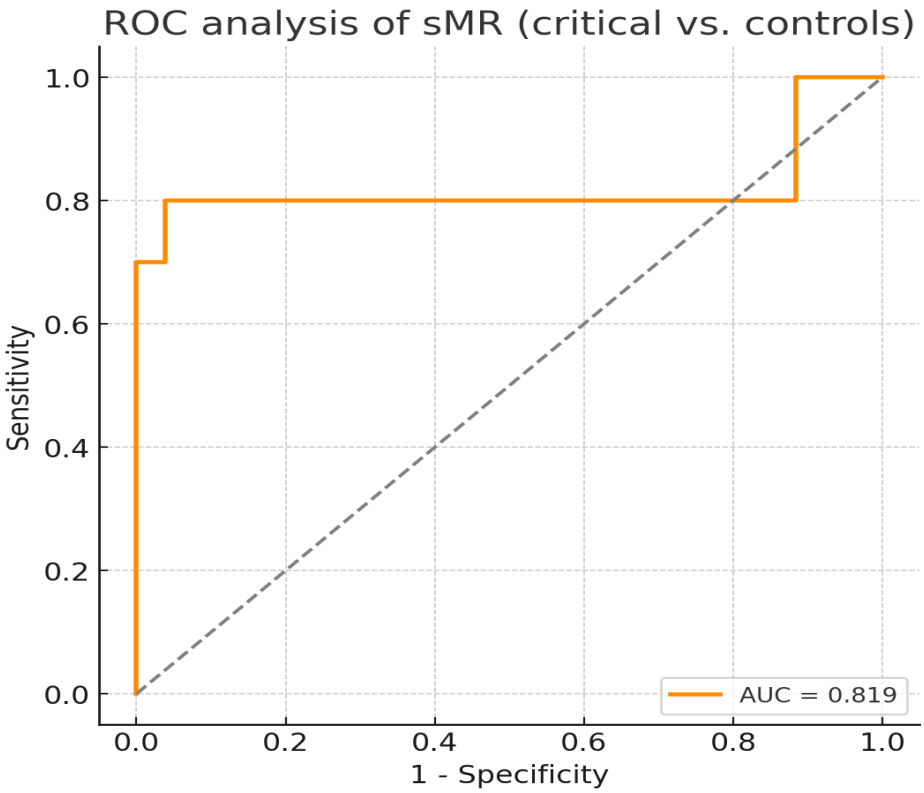


Figure 21. sMR ROC analysis in critical patients and controls



The results obtained in septic and critical patients demonstrated that procalcitonin (AUC = 0.649, 95% CI: 0.47-0.82) and CRP (AUC = 0.643, 95% CI: 0.47-0.82) had the highest discriminative performance. Leukocytes (AUC = 0.422, 95% CI: 0.22-0.61), presepsin (AUC = 0.471, 95% CI: 0.27-0.66) and sMR (AUC = 0.530, 95% CI: 0.34-0.72) showed lower diagnostic value. For sMR, the highest sensitivity (100%) but extremely low specificity (18%) was reported. Presepsin revealed high sensitivity (81%) but low specificity (36%). Procalcitonin (40%) and CRP (42%) showed low sensitivity but high specificity (100% and 90%, respectively). Procalcitonin and leukocytes achieved the highest positive predictive value (PPV = 100%). The negative predictive value (NPV) was highest for sMR (100%) and presepsin (65%). These findings are summarized in **Table 11** and illustrated through **Figure 22** to **Figure 26**.

Table 11. Diagnostic performance and reliability of biomarkers in septic and critical

Indicator	**Leukocytes (10^9/L)**	**CRP (mg/L)**	**Procalcitonin (ng/mL)**	**Presepsin (pg/mL)**	**sMR (ng/mL)**
AUC 95% CI	0.422 (0.22 - 0.61)	0.643 (0.47 - 0.82)	0.649 (0.47 - 0.82)	0.471 (0.27 - 0.66)	0.530 (0.34- 0.72)
Cut-off	29.75	34.33	2.33	147.97	112.63
Sensitivity %	11	42	40	81	100
Specificity %	100	90	100	36	18
PPV %	100	82	100	56	55
NPV %	53	61	62	65	100
p	0.4387	0.1515	0.1344	> 0.9999	0.7547

AUC - area under the curve, CI - confidence interval, PPV - positive predictive value, NPV - negative predictive value, Cut-off - cut-off point
Reference values - Leukocytes - age-matched, CRP - 0 - 5.0 mg/L, procalcitonin - 0.00 - 0.05 ng/mL,
Presepsin - 107.47 pg/mL, sMR - 152.81 ng/mL (95% reference interval, right-sided)

Figure 22. ROC analysis of leukocytes in septic and critical patients

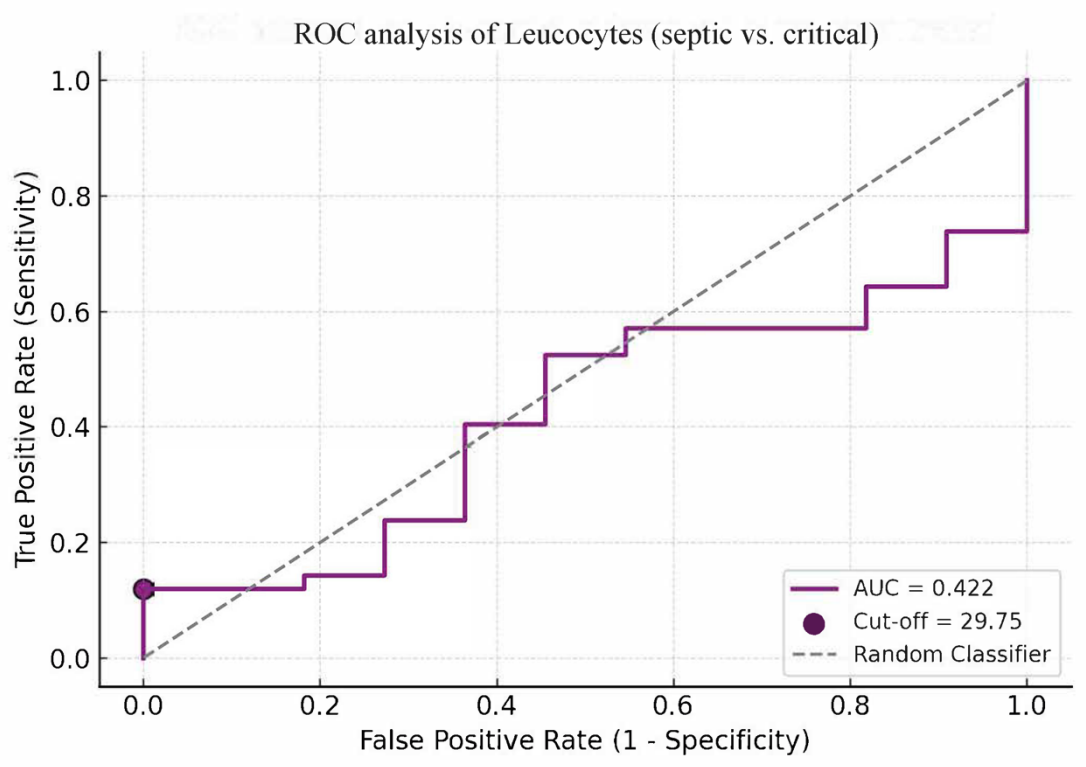


Figure 23. ROC analysis of CRP in septic and critical patients

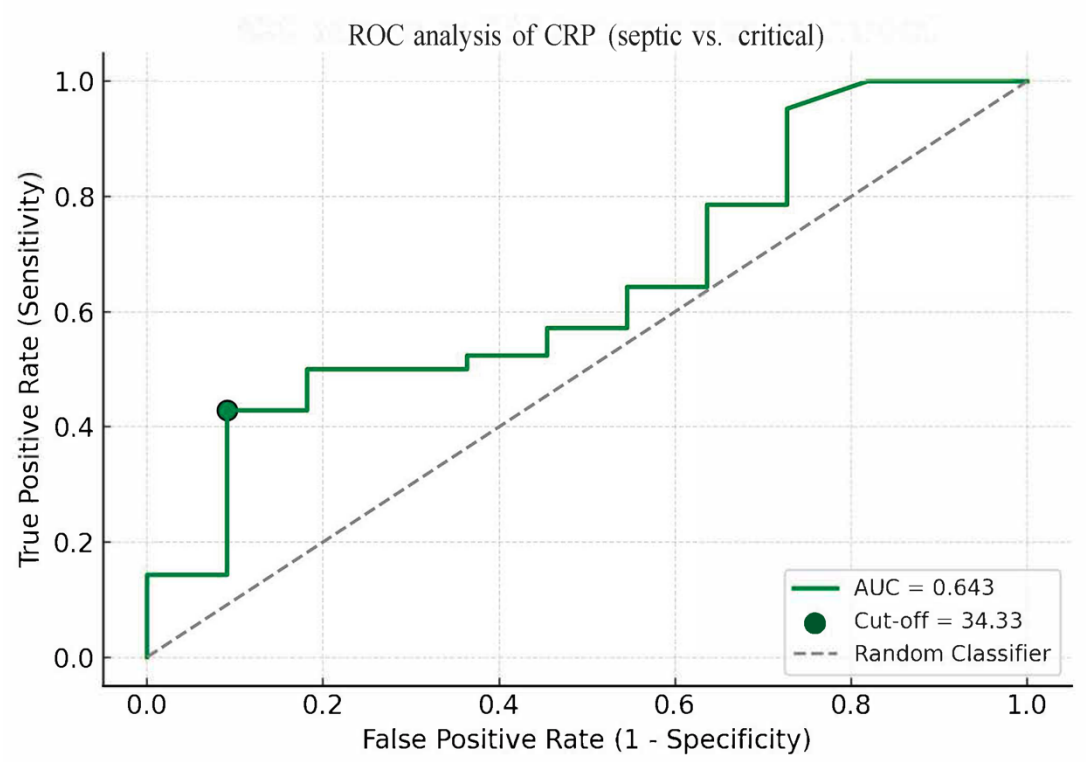


Figure 24. ROC analysis of Procalcitonin in septic and critical patients

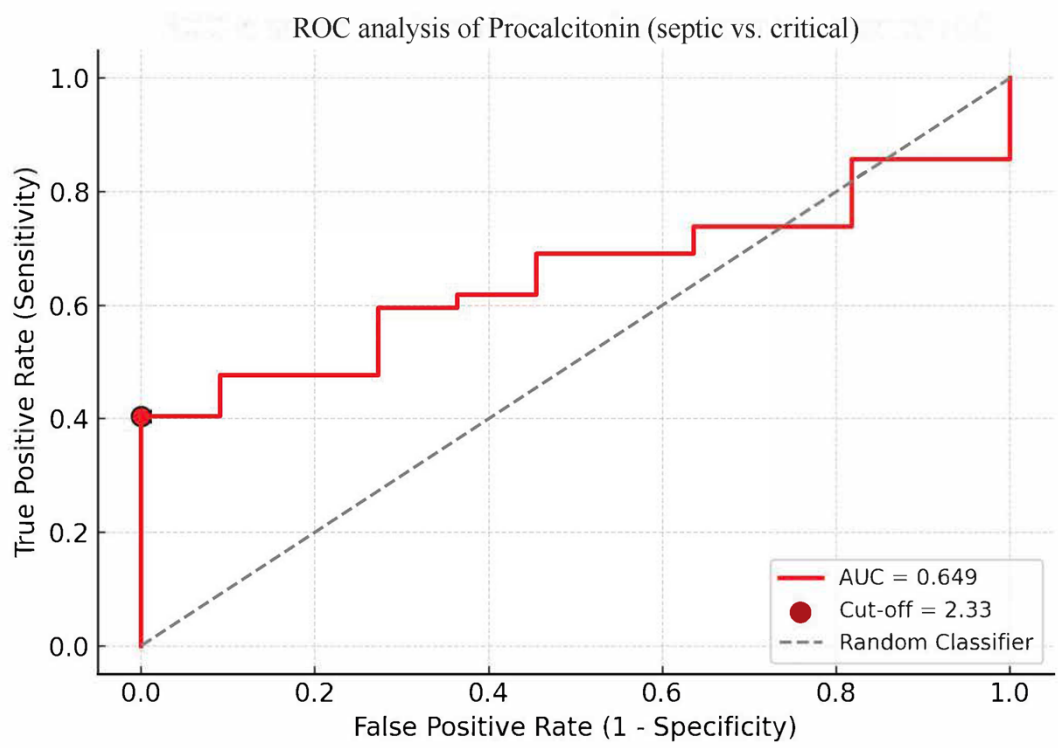


Figure 25. ROC analysis of Presepsin in septic and critical patients

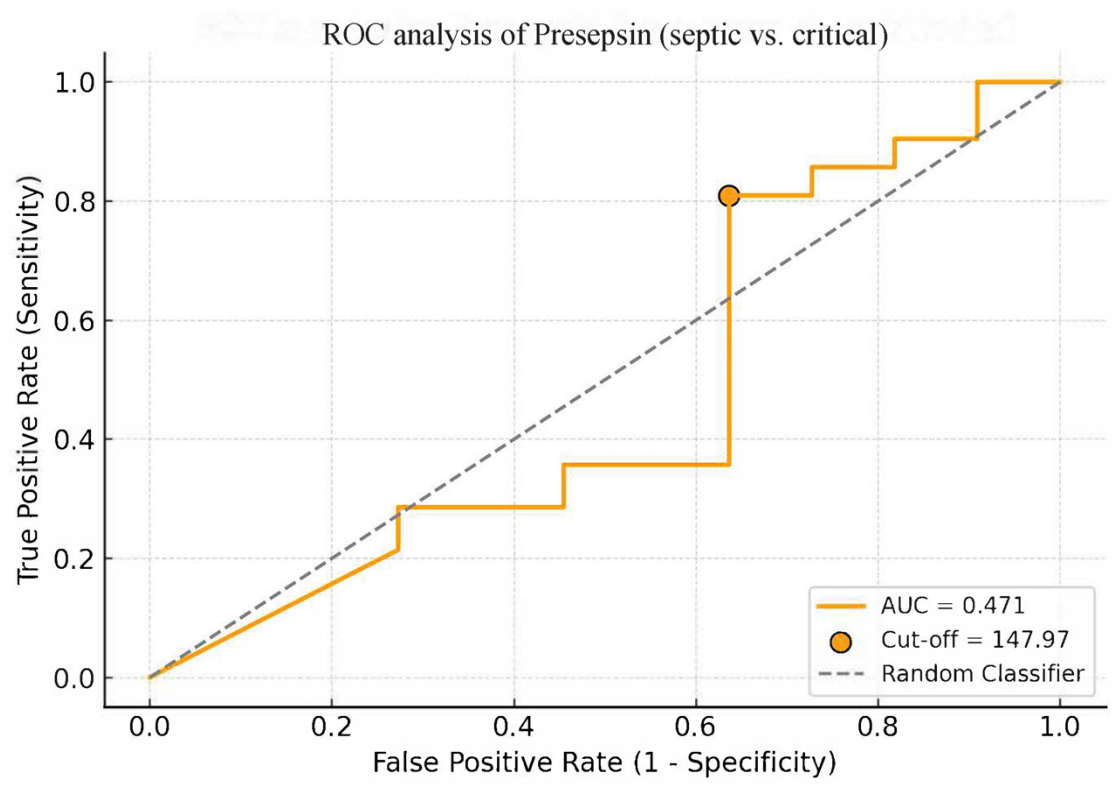
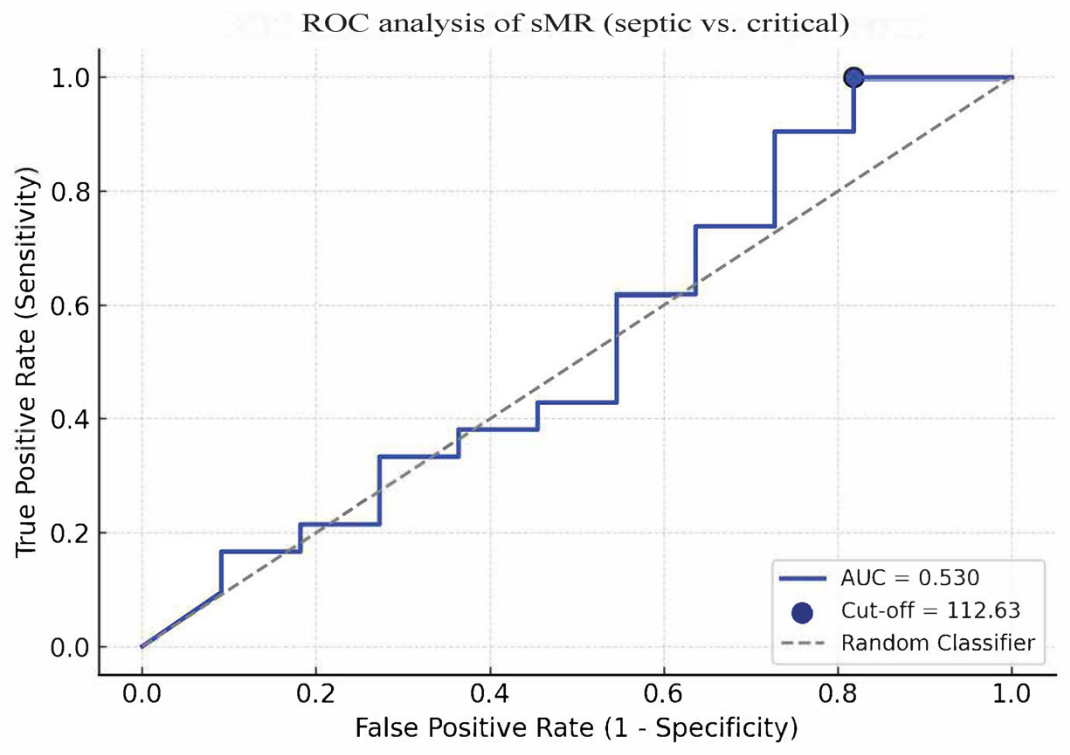


Figure 26. sMR ROC analysis in septic and critical patients



Combined biomarker analysis showed that procalcitonin combined with sMR demonstrated the highest diagnostic performance (AUC = 0.74), followed by procalcitonin + CRP (AUC = 0.71). Meanwhile, the CRP + presepsin combination (AUC = 0.56) reported the lowest discriminative potential, limiting its applicability. Among the analyzed models including three laboratory indices, **sMR + CRP + procalcitonin** (AUC = 0.78) showed the highest diagnostic performance for differentiating sepsis, providing the best discriminative ability among the evaluated combinations. The same are presented in **Figure 27** and **Figure 28**.

Figure 27. Multiple logistic regression of a combination of two biomarkers

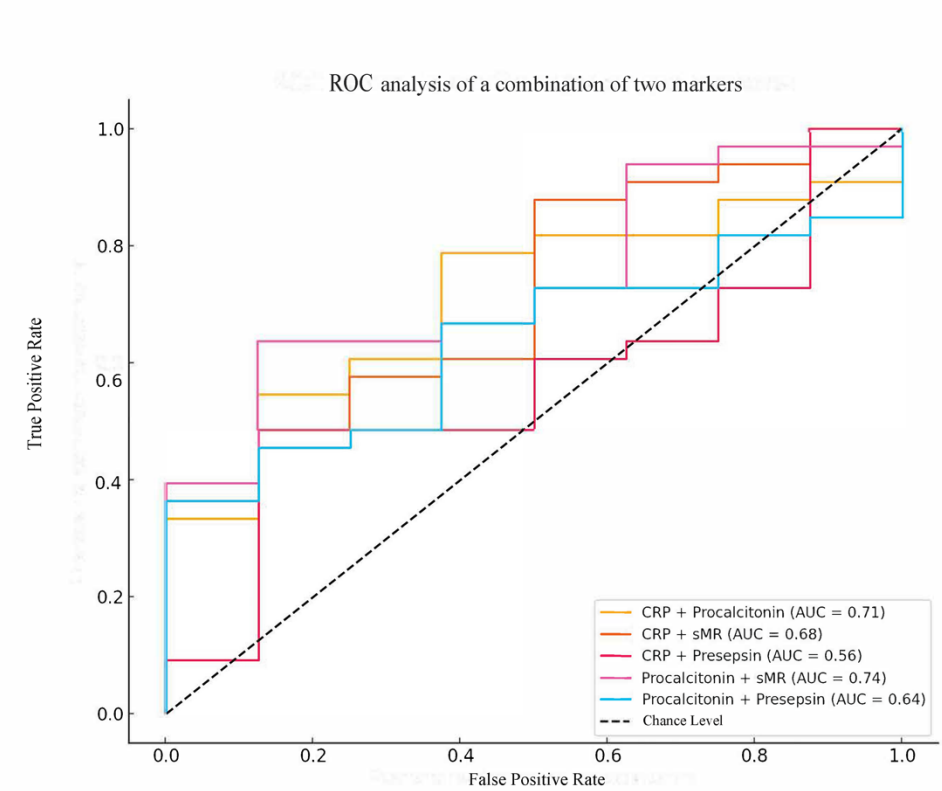


Figure 28. Multiple logistic regression of a combination of three biomarkers

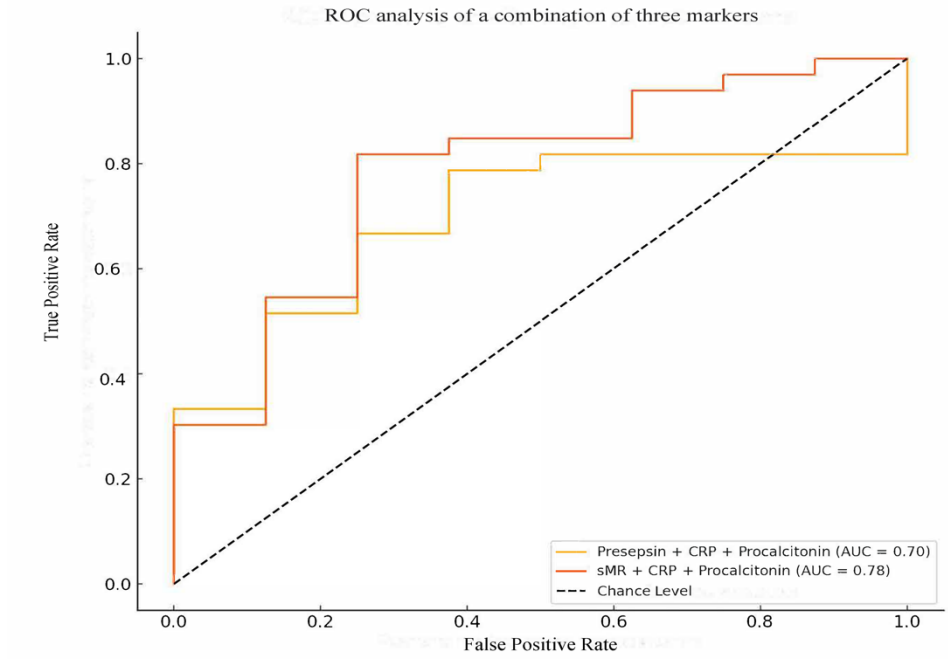


Table 12. Clinical characteristics and laboratory parameters of children under 2 months of age in the study group

Age (months)	Diagnosis	Type of complication	Stay (days)	Cause	Leukocytes (10^9/L)	CRP (mg/L)	Procalcitonin (ng/mL)	Presepsin (pg/mL)	sMR (ng/mL)
9 days	COVID-19 infection	no	6	COVID-19	9.92	2.35	1.19	3445.75	124.39
14 days	COVID-19 infection	no	6	COVID-19	12.68	0.12	3.90	273.30	192.21
1 month 24 days	Bronchiolitis	no	7	Unspecified	11.80	0.20	1.92	n/a	222.90
1 mo. 3 days	Bronchiolitis	no	7	COVID-19	9.95	0.60	2.82	353.00	193.85
27 days	COVID-19 infection	no	6	COVID-19	6.42	0.60	5.42	199.50	238.95
1 month 1 day	Enterocolitis	no	11	*Clostridioides difficile*	7.85	171.00	3.30	n/a	209.43
12 days	Omphalitis	no	8	*Enterobacter cloacae* - navel	25.32	86.26	1.49	190.00	237.85
15 days	Pneumonia	no	12	Unspecified	10.70	0.60	0.10	160.40	199.32
1 mo. 9 days	Tubulointersticial nephritis	Urosepsis	15	*E. coli* - uroculture and hemoculture	31.46	93.63	16.10	142.15	527.79
11 days	Pyoderma	Soft tissue abscess of upper limb	9	*Staphylococcus aureus* - wound discharge	22.18	5.73	0.09	n/a	n/a
1 mo.	Enterocolitis	no	20	*Pseudomonas aeruginosa* - feces	13.43	99.80	0.41	2534.00	856.38
29 days	Enterocolitis	no	5	Unspecified	13.11	107.83	30.10	129.50	266.83
23 days	Tubulointersticial nephritis	Urosepsis	8	*Klebsiella oxytoca* - uroculture	34.90	123.20	17.74	918.00	396.81
1 mo. 10 days	Pneumonia	Pleural effusion	17	Unspecified	8.22	0.60	0.10	183.50	125.78
1 month 4 days	COVID-19 infection	Pneumonia	8	COVID-19	15.82	55.20	25.70	399.00	309.88

n/a - missing value; Reference values - Leukocytes - age-matched, CRP - 0 - 5.0 mg/L, Procalcitonin - 0.00 - 0.05 ng/mL, Presepsin - 107.47 pg/mL, sMR - 152.81 ng/mL (95% reference interval, right-sided)

Table 12 shows the clinical and laboratory characteristics of the children under 2 months of age included in the study (15/53). Shorter hospital stay (5-6 days) was recorded mainly in COVID-19 infections without complications. The highest leukocyte counts (34.90 × 10^9/L) were recorded in a patient with urosepsis caused by *Klebsiella oxytoca*, while maximum CRP levels (171.00 mg/L) were observed in enterocolitis caused by *Clostridioides difficile*. COVID-19 patients demonstrated low procalcitonin levels (0.10-5.42 ng/mL). The highest concentrations of presepsin (2534 pg/mL) were found in a patient with enterocolitis caused by *Pseudomonas aeruginosa*. In children with bacterial infections (*Pseudomonas aeruginosa*, *Klebsiella oxytoca*), the highest sMR values (> 300 ng/mL) were reported, whereas in COVID-19-associated cases this marker ranged in the lower to moderate range (124-309 ng/mL).

Among septic patients, CRP ≥ 40 mg/L was recorded in 38.1% of cases, and 45.2% showed elevated PCT levels (2-10 ng/mL and > 10 ng/mL). Meanwhile, in 28.6% of children, PCT remained within the reference range (< 0.5 ng/mL). Only 9% of critical patients had CRP elevations ≥ 40 mg/L, supporting the notion that very high CRP values are more typical of sepsis than of other noninfectious conditions. A summary of these results is presented in **Table 13**.

Table 13. Comparative analysis of Procalcitonin and CRP values in septic and critical patients

PCT and CRP values in septic and critical patients		
Laboratory marker	**Septic n (%)**	**Critical n (%)**
PCT < 0.5 ng/mL	12 (28.6)	4 (36.6)
PCT 0.5 - 2 ng/mL	11 (26.2)	6 (54.4)
PCT 2 -1 0 ng/mL	10 (23.8)	1 (9.0)
PCT > 10 ng/mL	9 (21.4)	-
CRP < 5 mg/L	13 (30.9)	4 (36.4)
CRP 5 - 20 mg/L	8 (19.1)	4 (36.4)
CRP 20 - 40 mg/L	5 (11.9)	2 (18.2)
CRP ≥ 40 mg/L	16 (38.1)	1 (9.0)
Total *	**42 (100)**	**11 (100)**

*PCT - Procalcitonin, CRP - C-reactive protein, * - percentage ratio for the respective group; Reference ranges: CRP - 0 - 5.0 mg/L, Procalcitonin - 0.00 - 0.05 ng/mL*

V. DISCUSSION

Sepsis is one of the leading causes of death in children hospitalized in intensive care units. Nineteen years after the introduction of the historical IPSCC criteria, a new Task Force on Pediatric Sepsis Definitions has identified both the shortcomings of the original definitions and possible future solutions. The new Phoenix standards outperform those of the IPSCC, which until now was the only generally accepted classification of the disease. Developed from a clinical dataset covering over 3.5 million pediatric hospitalizations in ten hospital settings operating in independent health systems, they provide the first age-specific concept of sepsis in children as an infection-associated, life-threatening organ dysfunction.

Although information from different countries has been used for their detection, external validation of PSS will only be confirmed after further studies. One of the first implementations of the Phoenix criteria was presented in the study by Sanchez-Pinto et al. in 2025, highlighting their applicability and advantages in clinical practice in pediatric intensive care units in the United States.

Another significant change in the newly proposed definitions is the removal of SIRS as a clinical indicator of sepsis. This provides greater clarity and reduces diagnostic difficulties, especially in emergency departments where many children with fever and other abnormal physiologic indicators without sepsis meet the criteria for SIRS, e.g., febrile infants with bronchiolitis exhibiting tachycardia and tachypnea. Moving away from the widely accepted paradigm of SIRS over the past two decades represents not only a significant change but also a significant challenge, as conceptual evolution in medicine is inherently difficult.

Over the years, the understanding of the pathophysiology of sepsis has significantly deepened due to new data on host immune dysregulation. This process involves both the inability or inefficiency of the protective response to control infection at the site of pathogen entry and its direct involvement in the development of pathological changes. The main features of the immune response in sepsis include both excessive inflammation and suppression of immunity. These processes are observed in both adults and children. However, the features of pediatric sepsis are determined by age-specific immune responses. These regulate the complex interplay between inflammatory mediators, endothelial cell activation, complement and coagulation, and mechanisms of antigen presentation.

Outcome in sepsis has been shown to depend on the interplay between causative and host factors, including the virulence of the microorganism, the level of bacterial or viral load, and access to health care. Many pathogens have evolved, creating new strategies to enhance their reproductive success and survival in the host. However, mortality from sepsis is partly heritable. With rare exceptions (most commonly monogenic mutations), genetic susceptibility is usually not specific to an infectious agent, suggesting that similar mechanisms of immune dysregulation may be activated in different cases. It remains an open question whether these mechanisms represent endotypes, groups with coexpressed genes, and whether they can be influenced by targeted immunomodulation. This is an active area of research in sepsis science.

Although the presence of infection is a major criterion for the diagnosis of sepsis, not every infectious disease leads to its development. Unique to pediatric age groups is the fact that many primary immunodeficiencies initially present with sepsis, although such cases represent only a small proportion of pediatric sepsis. Furthermore, several congenital conditions have been found in extreme clinical phenotypes, such as the multisystem inflammatory syndrome associated with COVID-19 in children (MIS-C). Autoinflammatory polyorgan disease, proceeds atypically, resembling Kawasaki disease, Kawasaki-shock syndrome and toxic shock syndrome. The clinical picture is characterized by marked inflammatory activity, coagulation disorders, hypoproteinemia, and generalized lymphadenopathy, with no microbiologic evidence of a pathogenic agent. This is one of many examples showing how rare diseases can affect childhood health through increased susceptibility to infection or more severe course of infectious diseases.

- Etiologic pattern of illness in children with septic and critical conditions hospitalized in the PICU

The results obtained from this study provide detailed information on the etiological structure of illnesses in children with septic and critical conditions hospitalized in the PICU between June 2022 and January 2024, as well as the diagnostic value of the laboratory markers and clinical scales used. Global research data highlight the significant geographic variation in the incidence of paediatric sepsis and septic shock, necessitating an in-depth analysis of factors influencing the prevalence, diagnosis and therapeutic approach in different clinical and epidemiological settings. Disparities in incidence may be driven by access to health care, the availability of standardized protocols for early recognition and treatment, and the specificities of the microbiological spectrum and antimicrobial resistance in different regions. Although our sample is relatively small, the observed trends are consistent with global observations that demonstrate significant variation in the prevalence of sepsis. For example, in 2015, results from a study covering 128 pediatric intensive care units (ICUs) in 26 countries on six continents showed a sepsis prevalence of 8.2% (95% CI 7.6-8.9). In comparison, the prevalence in ICUs in South America was significantly higher at 25.9%, and the prevalence of septic shock reached 19.8%. In another US analysis based on electronic surveillance in hospital settings, the overall incidence of paediatric sepsis was found to be 0.69% (95% CI 0.67-0.71), and among hospitalised patients it was 2.8%. These results highlight the need for more in-depth studies, including in our region, to assess local factors that influence the incidence and clinical course of paediatric sepsis.

Similar studies have also revealed substantial variation in sepsis mortality among populations in different regions. For example, in Australia and New Zealand, a retrospective cohort study found an in-hospital lethality rate in EDs of 5.6% among children with sepsis, rising to 17.0% in patients with septic shock. In another European Childhood Life-Threatening Infectious Disease Study (EUCLIDS), the overall mortality from sepsis was 2.2% but reached 6% in patients admitted to the ED and 10% in those with septic shock. In contrast, this rate among hospitalized children with bacterial sepsis in Brazil remained high and relatively stable over time, at 20.5% for 1992-1996 and 19.7% for 2002-2006. Another study conducted in 2019 in 144 EDs in the same country showed a lethality rate of 19.8%. These data highlight the ongoing efforts of the medical community in addressing sepsis**.** In the present study, one case of lethality was recorded in a patient with underlying chronic disease. Possible factors contributing to this outcome may be both the severity of the underlying disease and the complexity of the septic process. Data from other large-scale studies have shown higher mortality, highlighting the need for further research on the impact of chronic disease on the clinical course and prognosis of paediatric sepsis.

Weiss et al. reported that the median age of those hospitalized with septic conditions in pediatric intensive care units was 3 years, which is consistent with our results. Age and the presence of comorbidity are among the most important risk factors for the development of sepsis. Its incidence remains strongly dependent on early life stages, leading to significant differences in epidemiological estimates when comparing studies including only paediatric intensive care unit or neonatal intensive care unit patients with population-based studies. An analysis covering 194 countries found that sepsis was responsible for 15% of neonatal deaths. In developing countries, this proportion is even higher, reaching 40% of cases in children under 28 days of age. The disease affects about 3 million newborns, with associated mortality rates steadily rising. Our study found that 13.2% of hospitalized patients were in the age range between 7 and 28 days. This result highlights the importance of the neonatal period as a high-risk period for the development of sepsis, which is consistent with observed trends in epidemiological studies. In our literature for the last 30 years, there is a paucity of data on sepsis in childhood, with a focus mainly on neonatal sepsis and the causative agents of nosocomial infections. Studies have shown that 32.93% of nosocomial infections were found in preterm neonates in Bulgaria between 2000 and 2011.

Data from the National Center for Infectious and Parasitic Diseases from 2023 indicate that the highest infectious morbidity in Bulgaria is associated with the respiratory and gastrointestinal systems. These data are consistent with our results of the highest relative proportion of those hospitalized with lower respiratory tract

infections (41.5%), followed by those with digestive system infections (11.3%). Similar analyses for respiratory illness as the leading cause of hospitalization were reported by Watson et al. (2003) in the USA, 37.2%, and by Martinot et al. in France, 43% (1997). A population-based study in England, spanning five decades, clearly showed that the incidence of hospitalizations due to invasive pneumococcal infections in children declined sharply after the introduction of immunization against *Streptococcus pneumoniae*. These results are supported by several recent studies showing a change in the aetiological profile of community-acquired sepsis, with *Neisseria meningitidis* no longer the leading causative agent as in previous periods. Despite these advances, vaccine-preventable diseases caused by these pathogens remain a significant global health problem.

A large-scale study on global and regional mortality associated with 33 important bacterial pathogens and 11 infectious syndromes (including bloodstream infections) in 2019 found 13.7 million deaths globally, with 7.7 million associated with the 33 bacterial pathogens studied. These pathogens, the most common of which include *S. aureus, E. coli, S. pneumoniae, Klebsiella pneumoniae and Pseudomonas aeruginosa*, were responsible for 54.9% of all sepsis-related cases in that year. Another large multicentre study in South Asia reported that in the age group 5-14 years, *Salmonella thyphi* was among the common aetiological contributors to mortality, and in younger age group, the leading pathogens in this regard were *S. pneumoniae*, followed by *S. aureus, Salmonella non-typhi, E. coli, K. pneumoniae* and *Neisseria meningitidis.*

In addition, Ikuta et al. found that lower respiratory tract infections had the highest disease burden, responsible for 4 million deaths in 2019. Another example is data from a large population-based study on chemoculturally proven sepsis in children under 17 years of age, which found differences among neonates, children with underlying diseases, and children with no evidence of comorbidity. These groups have distinctly different phenotypes in terms of causative agents, age, disease severity, and prognosis, with mortality rates ranging from 3% in patients without chronic disease to 7% in children with established disease. Within this study *Escherichia coli*, *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS) and *Streptococcus pneumoniae* were identified as the leading pathogens responsible for over 50% of cases. In this context, it should be noted that comorbidity in patients may mask or modify the symptoms of the pathogenic process, as well as increase susceptibility to systemic infections and the risk of adverse outcome due to compromised immune status. Consistent with these facts, a lethal outcome in our study was recorded in one child with genetic neuromuscular disease and etiologically undeciphered severe pulmonary infection.

According to the report of the European Project on Life-threatening Infectious Diseases in Childhood, a microbiological diagnosis was made in 47.2% of patients in the septic state. However, a study by Ribarova and colleagues found that two-thirds of cases of nosocomial infections in paediatric wards in Bulgaria lacked aetiological confirmation. The present study shows similar results to the European results cited above, with a specific causative agent identified in more than 40% of cases with infectious genesis. Our results demonstrate a predominance of bacterial pathogens over viral pathogens (55.6% vs. 44.4%), the latter represented mainly by SARS CoV-2 and Rotavirus. In contrast, a prospective study from Southeast Asia covering countries such as Indonesia, Thailand and Vietnam reported a predominance of viral infections in septic children (76%). In the sub-Saharan Africa region, malaria caused by *Plasmodium falciparum* is another significant cause of paediatric sepsis.

Bacterial species proven to cause infections associated with septicemic state from our data are *E. coli*, *Streptococcus pneumoniae, Staphylococcus aureus, Salmonella Group D* , and *Enterobaster cloasae.* These results are corroborated by Agyueman and Weiss, who also identified *E. coli*, *S. pneumoniae, S. aureus* and *E. cloasae* as etiological agents of septic conditions in childhood. Similarly for Bulgaria, in the 2-14 years age group, the most common infectious pathogens associated with adverse outcome were *S. pneumoniae, S. aureus* and *E. coli* associated with bacteraemia and lower respiratory tract diseases.

In this context, identifying the bacterial causative agents of septic conditions in children is a key aspect in optimizing the choice for effective therapy. Microbiological blood testing is the gold standard in the diagnosis of septic conditions, including in childhood, although in the paediatric population, due to its peculiarities, it may be unnecessarily performed in children with non-specific symptoms. Another limitation

is that haemocultures are often taken from patients who have already received treatment with broad-spectrum antibiotics, or the test is not performed at all on admission to a hospital ward. In addition, in young children, the volume of blood collected by venipuncture is significantly smaller (approximately 3 mL in children compared with 10 mL in adults), which further reduces the sensitivity of the haemoculture method. In addition, interpretation of a positive haemoculture when the blood sample is taken from a peripheral vessel should be done very carefully, especially when isolating representatives of the normal skin microflora, and if the patient has a central venous catheter in place, to rule out and confirm catheter-associated infection.

Accurate identification of the infectious agent is essential for adequate treatment and patient prognosis. The etiologic spectrum of bloodstream infections includes various types of pathogens, which are divided into three major categories: bacteria, viruses, and fungi, with bacteria occupying the largest proportion among them. Gram-negative bacteria are the leading causes of bacterial sepsis in the pediatric population. In a study conducted in Switzerland, the most frequently isolated pathogens from haemocultures were *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Enterobacter* spp. and *Burkholderia pseudomallei*. Among Gram-positive agents, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus suis*, *Streptococcus pneumoniae* and β-hemolytic streptococci predominated. In Bulgaria, 14% of all deaths associated with sepsis are caused by bloodstream infections. In the present study, one case of laboratory-confirmed bloodstream infection associated with *E. coli*, the primary focus of which was the urinary tract, which is consistent with evidence from the literature indicating urinary tract infections as a common cause of bacteremia and subsequent development of sepsis in children.

The use of the MALDI TOF Sepsityper (Bruker, Daltonics), as in the present study, allows identification of the infectious agent directly in the positive haemoculture in about 1 h from the time of blood sample positivity, before the causative agent is isolated on solid culture media, as suggested by standard haemoculture testing. The use of this automated system in routine laboratory practice makes it possible to dramatically shorten the time by 48 h for etiologic diagnosis and antimicrobial susceptibility testing. This approach has as its direct result the timely initiation of adequate etiologic therapy and the reduction of inappropriate and unnecessary empiric use of broad-spectrum antibiotics.

The importance of using such innovative diagnostic approaches is becoming increasingly important, especially in the context of increasing antibiotic use and the associated emergence and spread of antimicrobial resistance. Worldwide, it is estimated that there are about 2.4 billion children under the age of 18, with 27% of them under the age of 5. Global antibiotic use has increased significantly over the past two decades, and their use in children has contributed substantially to this growth. This is partly due to the high incidence of infectious diseases in childhood, as well as diagnostic challenges in young patients, leading to high rates of empiric antimicrobial prescribing. In neonatal intensive care units, antibiotics remain the most used medications. The trend of increasing antibiotic use was also observed in Bulgaria, from 49.49% in 2000 to 81.22% in 2011. This means that for every 100 children, 80 receive antibiotic treatment. In addition, in 2019, one in five deaths caused by an antibiotic-resistant bacterial pathogen was in a child under the age of 5.

It should be noted that over the past decade, there has been an alarming global trend of increasing antibiotic resistance among the most common causative agents of bloodstream infections in patients in pediatric intensive care settings, seriously threatening the effectiveness of antibiotic therapy. Multidrug-resistant *Enterobacteriaceae* (*K. pneumoniae* and others), *P. aeroginosa* and *A. baumannii* have been demonstrated with increasing frequency in hospitalized patients, including critically ill patients under 18 years of age. In this sense, the increased resistance of pathogens among the most frequent causative agents of bacteremia in pediatric intensive care patients seriously threatens the feasibility of the currently used antimicrobial therapy algorithms. Multiple studies of the problem among injured children have demonstrated not only the prevalence of these multidrug-resistant bacteria, but also the higher mortality rates (over 50%) of the invasive infections with which they are associated compared with infections caused by susceptible strains of the same bacterial species. Moreover, hospital length of stay has been identified as an independent risk factor for bacteraemia caused by multidrug-resistant bacteria. Other studies have found that carbapenem resistance is independently associated with death, which is likely associated with a delay in adequate therapy

(due to resistance to recommended empiric therapy) until identification of the pathogen and its sensitivity to antibiotics is obtained.

In summary, inappropriate use and overuse of antimicrobial drugs, as well as easy access to them, are among the most important factors responsible for the development of microbial resistance. The results of the present study showed that a very high relative proportion of patients in group I (more than 30%) received antibiotic treatment within the last 7 days before admission to the DOH, which negatively affected the timely and accurate diagnosis.

In addition to patients with confirmed or suspected infection, critically ill children whose conditions are not related to infections or infectious diseases but require intensive treatment and a multidisciplinary approach are often hospitalized in intensive care units. In our study, a specific cohort of 11 such patients with life-threatening conditions of various etiologies were analyzed, in whom continuous monitoring of vital signs and complex therapy was required to stabilize vital functions.

According to a study in Australia by Ibiebele et al. (2018), diabetic ketoacidosis is one of the most common causes of hospitalization in pediatric intensive care units, ranking second only to traumatic injuries. An analysis covering 46 centres in 19 countries confirmed that trauma and injury is the leading cause of hospitalisation among children with critical conditions in all sociodemographic groups examined. Our results show that in patients with SIRS of non-infectious origin, intoxications and diseases related to endocrine and metabolic disorders, such as insulin-dependent diabetes mellitus with initial ketoacidosis, account for the largest relative proportion. These data are consistent with the results of international studies.

- Scoring systems

The timely identification of a child with sepsis among the many febrile patients presents a significant challenge. Nonspecific symptoms in the early stages of the disease make it difficult to define and diagnose, even for experienced physicians. In the present study, the prognostic value of various risk scales in children with septic and critical conditions was evaluated. The analysis focused on their predictive accuracy, sensitivity and discriminatory ability. The results of the study showed that PRISM III had better predictive ability compared to PELOD-2 in children with septic and critical conditions (62.3% vs 58.5%). However, both scales showed similar numbers of false negatives in identifying patients with complications, which may lead to lack of timely intervention and underestimation of the patient's overall condition. Furthermore, over 50% of patients with real complications were missed by the pSOFA model, indicating low sensitivity in identifying high-risk cases with sepsis. In contrast, PSS demonstrated better performance, being able to identify a significantly higher proportion of patients with complications.

An additional aspect of the analysis shows that PELOD-2 has limited performance, manifested in low discriminatory ability and sensitivity. This contradicts the results of the El-Nawawy et al. study conducted in an African population, where PELOD-2 reported significantly higher AUC (0.90) and sensitivity (76%) values. A possible explanation for this contrast is that the initial development and validation of PELOD-2 were based on information from nine paediatric intensive care units in France and Belgium, which, together with the relatively small data volume, may limit its applicability in other geographical and ethnic groups. Therefore, further validation of the scale in different regions of the world is essential to increase its reliability and applicability.

In the context of our study, the PRISM III demonstrated a predictive value comparable to that reported by Jhamb et al. (AUC 0.701). These data suggest that PRISM III remains a reliable predictor in diverse populations, although its discriminatory ability is moderate. With respect to pSOFA, the high specificity (over 80%) is particularly useful in identifying patients without complications, but the low sensitivity indicates that the model misses a significant proportion of patients with actual organ dysfunction among those hospitalized. In view of these results, the use of additional indicators, e.g. laboratory markers of inflammation such as CRP, procalcitonin, presepsin, may reduce false negative cases and make the model more reliable for predicting complications in septic patients.

Among the prognostic tools analyzed in this study, Phoenix Sepsis Score stands out as the most effective prognostic scale for identifying septic patients with complications. With a sensitivity value of 80.0% and an AUC of 0.736, the PSS demonstrated high reliability in correctly classifying patients with suspected infection. This makes it an extremely valuable method for optimizing the diagnostic and therapeutic process in clinical practice. The observed results are consistent with the study of Wolf et al. 2024 where PSS successfully classified the risk of mortality in children with oncohematological diseases admitted to intensive care units with suspected infection. In another retrospective study conducted in nine paediatric intensive care units in the USA, it was confirmed that the Phoenix criteria identified a significant proportion of high-risk patients not accounted for when applying the IPSCC definition.

In addition, the current definition of sepsis uses validated indicators of organ dysfunction to assess the risk of sepsis and septic shock. Analysis of data by Sanchez-Pinto et al. (2025) showed that the risk of in-hospital death in children with PSS ≥ 2 was eight times higher compared with patients with suspected infection who did not meet these criteria. Furthermore, in low-income countries, over 25% of children with PSS ≥ 2 died in hospital. These results highlight the potential of the Phoenix criteria for pediatric sepsis, demonstrating promising opportunities for earlier identification of at-risk patients and prediction of disease severity.

Larsen and Workman note that synthesizing a complex multi-organ process into a simple and valid numeric scale is extremely useful. They emphasize that the thorough, consensual, and data-driven approach, incorporating information from both low- and high-risk countries and different health systems, makes the new definitions more comprehensive and applicable to a broader population-something that is missing from the 2005 criteria. However, the authors point out that the Phoenix standards are not intended for early recognition of children at risk for sepsis and septic shock, nor for screening for the disease. This highlights the need to develop effective methods for early detection of paediatric sepsis built on the same evidence-based and evidence-informed approach as the Phoenix criteria. Such a strategy would facilitate pediatricians in the care of children at risk for the disease and its associated complications.

In this context, because of the poor calibration of the PSS prognostic model, use in its original form in our population necessitated further reevaluation. It has been shown that when the model has sufficient discriminatory ability, recalibration can improve its accuracy and performance in a specific population. In view of these facts, adaptation of the PSS by the calibration intercept (λb) and calibration slope (βb) resulted in good model fit and adequate prediction of patients' risk of developing a complication.

The trends observed in the present study reflect the complex interaction between clinical factors and patient condition. In addition, the role of chronic diseases, the need for mechanical ventilation and the length of hospital stay are key parameters that require in-depth analysis. Although the presence of chronic disease did not show statistical significance in the Cox analysis, the results demonstrated a trend toward an increased risk of developing complications by more than 70% in hospitalized patients with underlying diseases. This conclusion is supported by clinical observations based on the recorded lethal outcome in the present study, a patient with severe pulmonary infection of microbiologically unconfirmed etiology, against a background of congenital neuromuscular disease. The results highlight the importance of chronic conditions as a potential risk factor for complications. They are also corroborated in the study by Kortz et al. cited above, with the most affected patients with critical conditions having two or more comorbidities.

Regarding mechanical ventilation, although it did not show a statistically significant influence in the present study, the results suggest a trend towards a reduction in risk. It is important to note that the proportion of patients requiring mechanical ventilation in our study (less than 20%) was significantly lower compared with reported values in other studies, 23.3% and 68.8%, respectively (242,243). Discrepancies may be due to specific characteristics of the study population or different criteria for the application of mechanical ventilation. Therefore, these findings highlight the need for further analyses to clarify these variations and their impact on prognosis.

Length of hospital stay represents another important prognostic factor. In the present study, the mean number of days of ICU stay was 8 days, which significantly exceeds the reported values in Portugal and Egypt (3 and 4 days, respectively). The discrepancy may be due to various factors, such as the severity of clinical

cases in different populations or the specific characteristics of the health systems in which the studies were conducted. For example, according to Gonçalves et al. the shorter length of stay in Portugal could be explained by the higher proportion of patients recovering from surgical interventions that usually require a short hospital stay. These differences highlight the need to adapt therapeutic strategies to local conditions and patients' clinical characteristics. Furthermore, when comparing data between different health systems, it is essential to consider structural as well as organizational factors that may influence both the length of hospitalization and the overall prognosis of critically ill patients.

- Laboratory markers of inflammation

The major challenge clinicians face in diagnosing sepsis is the absence of a universal and highly reliable biomarker or method that provides rapid identification of the disease. Although CRP and procalcitonin have long been used as laboratory indicators of inflammation and sepsis, as well as the more widespread use of procalcitonin during COVID-19, they have not been implemented as routine diagnostic practice in most hospital settings in this country.

To use more precise diagnostic indicators, numerous studies have focused on the evaluation of various biomarkers in patients with suspected sepsis. As early as the 1980s and 1990s, leukocyte counts were used as a primary indicator of infection, but with the development of analytical technologies, evidence of its limitations is accumulating. In one of these studies, Marik et al. found that leukocyte count had very low predictive value, with an AUROC of only 0.52. The present study confirms these observations, further highlighting their weakness as a stand-alone diagnostic marker. This calls for the development of more accurate and specific biomarkers to provide earlier and reliable identification of high-risk patients.

Like the present study, De Rop et al. found that patients with complicated infections tended to have higher blood CRP levels. However, they reported cases of severe disease with CRP levels < 5 mg/L, highlighting its limited diagnostic significance in certain clinical situations. In support of these observations, in the study by Pontrelli et al. PCT demonstrated moderate diagnostic performance for sepsis in neonates with suspected infection at cutoff values of 2.0-2.5 ng/mL. In our study, 3 of 7 neonates with symptoms of sepsis showed elevated PCT values, but only one of them also had CRP values outside the reference range. This result highlights the differences in diagnostic sensitivity of the two biomarkers and their individual roles in identifying septic conditions.

The cut-off values of procalcitonin and CRP found in our study were in accordance with internationally accepted threshold limits for sepsis and severe bacterial infections in children, confirming their applicability as diagnostic indicators in the study cohort. However, there are conflicting results regarding their independent diagnostic reliability. For example, Downes et al. defined PCT testing as insufficiently sensitive and specific for self-diagnosis. Similar results were observed in the study by Arkader et al. in which CRP failed to differentiate between the conditions of sepsis and noninfectious SIRS in children, with a reported AUC value of only 0.54. In this context, our results suggest that both CRP and PCT have limited discriminatory power in disease confirmation. This highlights the need to combine these biomarkers with additional laboratory indicators of inflammatory response to improve diagnostic performance in septic patients.

Similar findings were reported in a meta-analysis published in 2015 showing that presepsin is a useful laboratory marker for the diagnosis of sepsis but is insufficient to confirm or rule out the condition when used alone. In the context of the present study, the reference limits reflect the maximum values measured in 95% of healthy individuals, only 5% of whom may have higher levels of the relevant biomarker. These values serve as a guide to normal physiological variations and assist in distinguishing potentially pathological indicators. At the same time, the cut-off values identified play a key role in diagnostic interpretation as they represent the threshold above which the test is considered positive. However, with low AUC and specificity values, their analytical significance remains limited. In the present study, presepsin and sMR showed low diagnostic performance, limiting the possibility of their independent application in clinical practice.

On the other hand, presepsin demonstrated a better balance between sensitivity (81%) and specificity (36%), suggesting that it can detect a significant number of ill patients, but at the same time is characterized by a high false-positive rate. However, the sMR shows the maximum sensitivity but low specificity (18%), demonstrating that all sick patients are correctly identified but many healthy individuals are also classified as positive. This substantially reduces its effectiveness as a stand-alone diagnostic marker. The identified limitations associated with low specificity and high false-positive rates necessitate combining presepsin and sMR with other biochemical indicators with better discriminative ability.

In this context, the high negative predictive value of sMR (100%) suggests that it may be useful for excluding a diagnosis but not for confirming it. Although some studies, such as that of Hassuna et al. suggest that sMR and presepsin can differentiate sepsis from noninfectious SIRS in critically ill children, our results do not support this hypothesis. A major drawback remains the lack of universally defined reference and cut-off values, making it difficult to unambiguously distinguish between positive and negative results. This highlights the need for standardization and further studies to define optimal age-specific cutoffs to increase the diagnostic reliability of these laboratory indices in clinical practice.

Our analyses showed in direct comparison between septic patients and Group II that CRP and procalcitonin had better potential to differentiate sepsis from noninfectious inflammatory conditions, whereas leukocytes, presepsin, and sMR demonstrated lower diagnostic specificity and limited performance in this differentiation. Furthermore, boxplot analysis revealed that sMR and presepsin accounted for the large variability in critical conditions, making it difficult to differentiate between septic and noninfectious SIRS patients. These data are consistent with the study by Song et al.

These results demonstrate the importance of combining biomarkers to improve the accuracy of identifying infectious conditions. A few reports have reported excellent diagnostic performance when combining CRP and procalcitonin. In our study, combining CRP and procalcitonin confirmed its clinical relevance as a well-established diagnostic method. In addition, the pattern of sMR, CRP and procalcitonin demonstrated the best performance, suggesting that sMR is the better innovative marker in our experimental setting and contributes to improved analytical value in the demonstration of sepsis. These data highlight the potential of sMR as an innovative biomarker that can complement standard laboratory indices and improve their diagnostic accuracy.

Furthermore, studies suggest that combining screening tools and clinician judgment significantly improves sensitivity and specificity compared with the use of diagnostic-guidance tests alone in sepsis. However, detection of the disease remains difficult due to insufficient training and awareness among both medical professionals and researchers and the public. Lack of adequate recognition by parents of early signs of the disease often leads to delayed seeking of medical care, which delays hospitalization and worsens the prognosis of patients. Analyses of these factors highlight the need for continued education and refinement of diagnostic algorithms to improve the timely identification and treatment of childhood sepsis.

- Treatment, prognosis and development horizons

The need to develop more specific methods to differentiate septic conditions is particularly important to reduce unnecessary antibiotic use among children in whom the infectious process is not confirmed. It is important to note that observational data linking lower mortality to rapid administration of antimicrobials (within 1 hour of sepsis identification) are based primarily on patients in shock. In clinical practice, however, emergency departments and inpatient units frequently encounter children in whom sepsis is only one of several possible diagnoses. In view of the potential adverse effects of unnecessary and excessive antibiotic treatment and the long-term risks to individual and public health, the implementation of a rational antibiotic policy in patients with suspected sepsis represents a key strategy for improving the quality of the treatment process and patient care.

In addition to early diagnosis, a key factor in improving survival is optimizing drug therapy. In this context, the FEAST study demonstrated increased mortality associated with the administration of rapid bolus

infusions in children with life-threatening infections in income-constrained countries. These results highlight the importance of hyperchloremic acidosis as well as progressive respiratory and neurological dysfunction as factors associated with increased mortality after administration of bolus infusion therapy. A number of randomized controlled trials are being conducted to improve therapeutic strategies for pediatric sepsis. These include PROMPT Bolus (NCT04102371), which investigated the effect of different infusion solutions during resuscitation, and SHIPSS (NCT03401398), focusing on the potential therapeutic effect of hydrocortisone. Despite these efforts, significant questions remain regarding the optimal timing and amount of infusion therapy, the appropriate titration of inotropes and vasopressors, and the role of immunosuppression in the treatment of affected children.

In addition to the treatment of patients, increasing attention of the medical community is focused on minimizing long-term sequelae in patients undergoing intensive care. This is necessitated by increasing evidence of lasting physical, cognitive and psycho-emotional deficits following severe illness and intensive therapy. Data from Carlton et al. (2022) show that 19.4% of dehospitalized children who experienced sepsis developed lasting complications. In this context, the results of the present study show a significantly lower percentage - less than 5% of those hospitalized with sepsis required prolonged medical monitoring and specific care. These differences are likely due to certain characteristics of the study population, as well as different intensive care and subsequent rehabilitation strategies.

Published prognostic models and rates integrate clinical, laboratory, and physiological parameters, as well as novel biomarkers, to varying degrees. The condition rating scales are primarily aimed at predicting early clinical deterioration, mortality, organ dysfunction and length of hospital stay. Examples of models and rates used in children with sepsis include various versions of the pediatric early warning rates, pSOFA, PELOD-2, and others. In addition, the Pediatric Sepsis Biomarker Risk Model (PERSEVERE) developed by Wong and collaborators uses five biomarkers to predict 28-day overall mortality and persistent organ dysfunction in children with septic shock. Compared with models predicting short-term outcomes, there is a distinct lack of algorithms that predict long-term outcomes in children, including health status and functional impairment affecting quality of life after intensive care. A 2019 study examining predictors of general health in 790 children who experienced sepsis an average of 31 days after discharge found that older age, immunocompromised status, septic shock, and prolonged hospital stay were associated with poorer social and physical well-being. Scoring systems such as PELOD-2 showed no association with reduced standard of living, suggesting that the models most used to predict short-term outcomes may not be effective in predicting long-term outcomes. In another study conducted as part of the *Life After Paediatric Sepsis Evaluation (LAPSE*) study, a subgroup of children who had experienced community-acquired septic shock was analysed. In it, the *PERSEVERE* biomarker model failed to predict quality of life after intensive treatment in patients followed up for three months after discharge from hospital. These results highlight the limitations of existing prognostic models in assessing the long-term sepsis sequelae and the need for additional research that integrates biomarkers and functional indicators to more accurately assess recovery.

With this aspect in mind, Gilholm and collaborators developed an artificial intelligence (AI)-based method that uses data from intensive care units to predict long-term cognitive outcomes in patients who have experienced critical conditions. This algorithm analyzes children's cognitive ability to meet minimum school requirements over a six-year follow-up period. The study included different groups of children treated for sepsis and found that socioeconomic status, comorbidities, and severity of illness were predictors of failure to meet minimum school standards. Although long-term outcomes are increasingly recognized as a priority research area, most published models have limitations, including small sample size, short follow-up period, and lack of external validation. Furthermore, these methods are rarely applied in real-world clinical settings. To improve the accuracy of prediction, integration of large and diverse clinical databases with predictive models is needed to allow individualized risk assessment. This would facilitate timely decision making, especially if these algorithms were integrated into real-time monitoring systems.

Despite their potential, the literature evidence for the effectiveness of these approaches in real-world clinical settings is limited. The development of predictive approaches that are proven useful in practice remains

a key scientific and clinical need. This is particularly important for the development of strategies aimed at improving long-term outcomes in patients at high risk of adverse sequelae after experiencing severe sepsis or another critical condition.

In this context, it should be noted that the construction of paediatric intensive care units is key to improving overall survival of paediatric patients in Bulgaria. According to the report of the National Health Strategy of Bulgaria until 2030, the main reasons for untimely medical care and development of permanent disabilities in critically ill patients are limited access to health care in remote locations, lack of qualified personnel and equipment. There are 7 university-based paediatric intensive care units in Bulgaria, five of which have the status of PICU. Despite the existence of these specialized facilities, more than 25% of hospitalized children were brought from facilities with a lower level of competence. This highlights the need for sufficient availability of material resources and qualified medical staff concentrated in intensive care units.

It is important to note that our country has not yet adopted a protocol for the initial assessment of children in intensive care. Establishing clear criteria for the admission of critically ill patients will ensure early identification of life-threatening conditions, optimization of the therapeutic approach, reduction of long-term sequelae and reduction of child mortality.

Study Limitations:

The studies conducted cannot be considered fully representative of the general population as the study was limited to a specific target group and the innovative biomarkers studied were analyzed for experimental purposes. Larger, multicentre and prospective studies involving a broader and heterogeneous population are needed to assess the diagnostic value of the methods investigated.

VI. FINDINGS

1. Infections of the lower respiratory tract, gastrointestinal tract and nervous system were the leading causes of sepsis in the study group.

2. A high relative proportion of microbiologically confirmed infections in septic conditions was demonstrated, with a predominance of bacterial causative agents. The most frequently documented pathogens were *E. coli, S. pneumoniae*, and *S. aureus, which* correlates with global data on the etiology of sepsis in children.

3. Among the four clinical scales studied, the Phoenix Sepsis Score showed the best overall predictive value and the highest reliability in predicting complications in children with septic and critical conditions.

4. The sMR and presepsin show considerable variability, highlighting the need for further validation in larger cohorts to confirm their diagnostic and prognostic value.

5. procalcitonin and CRP are confirmed as the most reliable biomarkers for sepsis, while sMR and presepsin may be useful as additional markers to aid diagnosis.

6. Simultaneous testing of sMR, CRP and procalcitonin is a useful diagnostic approach in the clinical evaluation of patients with sepsis.

VII. CONTRIBUTIONS

1. This study is one of the few in the current literature to examine in detail the etiologic spectrum of disease in children with septic and critical conditions hospitalized in the intensive care unit.

2. The role of modern microbiological methods in proving the microbial causative agent of septic conditions, limiting empiric antibiotic use and initiating timely etiological treatment is confirmed.

3. For the first time, a comparative evaluation of four international scoring scales for critical and septic conditions in children hospitalized in the intensive care unit.

4. New predictive biomarkers for the diagnosis of sepsis in children were evaluated.

5. Validated the combined use of CRP and procalcitonin as a reliable laboratory approach for the diagnosis of sepsis in the pediatric population.

6. The results obtained and analyzed in this study are the basis for the establishment of an effective model for the diagnosis and treatment of septic conditions in childhood.

VIII. CONCLUSION

The recognition of sepsis in children, including those in critical condition, is not a well-defined and well-studied process both in our country and worldwide. This dissertation is dedicated to an in-depth study of sepsis and critical conditions in children treated in the intensive care unit, focusing on the clinical profile, early diagnosis and prognosis of these patients. Based on the results, a few scientifically based conclusions are formulated that have practical relevance for improving the diagnostic process and therapeutic management of pediatric patients.

The study found that the highest relative proportion of hospitalized patients was observed in the age group 13-48 months, which highlights the specific features of the immune system in this age category and its increased vulnerability to severe infections. In the group of septic patients, lower respiratory tract infections were found to be predominant, whereas in children with non-infectious SIRS, intoxications were the leading cause of hospitalization. This observation highlights the need for increased attention to prevention and early recognition of these conditions in clinical practice.

The study revealed a high relative proportion of microbiologically confirmed infections (over 40%), with bacterial causative agents outnumbering viral agents. The most isolated etiologic agents in septic patients included *Escherichia coli*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, consistent with global trends in the prevalence of septic infections in childhood. Knowledge of the microbial spectrum of the causative agents of sepsis allows for etiologically targeted antibiotic therapy, which is more effective than the empirical approach and reduces the risk of antibiotic resistance.

In addition, the application of modern diagnostic methods provides faster and more accurate identification of infectious agents, allowing timely initiation of therapy, a personalized approach to each patient and reducing the risk of long-term complications. In this context, rapid microbiological techniques, such as PCR and MALDI-TOF mass spectrometry, represent promising tools to improve the diagnosis of childhood sepsis.

Another significant achievement of the study is the comparative analysis of scoring scales for the evaluation of septic conditions in children. The results show that the Phoenix Sepsis Score outperforms the established PRISM III, PELOD-2 and pSOFA scales in terms of sensitivity and specificity for predicting the septic process and associated complications.

One of the significant results of this dissertation is the proven clinical value of modern biochemical markers of inflammation. CRP and PCT have been found to remain essential markers in the diagnosis of sepsis, but their limited specificity highlights the need for additional methods for more accurate and timely recognition of the disease. In this context, experimental biomarkers such as presepsin (sCD14-ST) and soluble mannose receptor (sMR; sCD206) show potential to improve the early diagnosis of sepsis, but their clinical value requires further studies and validation in larger cohorts.

The combination of the above innovative laboratory markers and methods could significantly improve the diagnosis and treatment of severe septic and critical conditions in infancy and childhood and potentially reduce associated mortality. New data could contribute to greater effectiveness of antibiotic treatment and, subsequently, to the prevention of septic conditions in children. Finally, standardized personalized treatment protocols that combine modern microbiological and biochemical diagnostic approaches are needed to improve survival and reduce complications in children with septic and critical conditions. In addition, future research should focus on the development and validation of new biomarkers for early diagnosis that build on existing methods and allow more accurate risk stratification.

IX. THESIS-RELATED PUBLICATIONS

- **Hadzhieva-Hristova A**, Stoeva T, Iotova V. Innovative markers of inflammation in children with septic and critical conditions. *Pediatrics*. 2023; 4: 7-10

- **Hadzhieva-Hristova A**, Stoeva T, Niyazi D, Iotova V. Clinical profile and etiological structure of diseases in children with septic and critical conditions hospitalized in a pediatric intensive care unit in Bulgaria: a single-center study. *Scripta Scientifica Medica*. 2025;55(2):[Online first]. Available from: http://dx.doi.org/10.14748/ssm.v55i2.10069

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