Prognostic impact of increased serum presepsin concentrations on sepsis outcome

Aliu-Bejta, Ajete

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This doctoral study was conducted at the Clinic of Infectious Diseases in Pristina, University Clinical Center of Kosovo, and the University Hospital for Infectious Disease “Dr. Fran Mihaljević” in Zagreb, Croatia.

Mentor: Professor Bruno Baršić, MD, PHD

Co-mentor: Professor Shemsedin Dreshaj, MD, PHD

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ABBREVIATIONS

ACCP:	the American College of Chest Physicians
AIDS:	Acquired immune deficiency syndrome
AKI:	acute kidney injury
ALBIOS:	Albumin Italian Outcome Sepsis trial
ALT:	alanine aminotransferase
APACHE:	Acute physiology and chronic health evaluation
APS:	Acute physiology score
ARDS:	acute respiratory distress syndrome
AST:	aspartate aminotransferase
ATS:	the American Thoracic Society
AUC:	Area under the ROC curve
CARS:	compensatory anti-inflammatory response syndrome
CD14:	cluster of differentiation 14
CLP:	cecal ligation and puncture
CNS:	central nervous system
COPD:	chronic obstructive pulmonary disease
CRP:	C-reactive protein
CSF:	cerebral-spinal fluid
DAMPs:	Danger-associated molecular patterns
DIC:	disseminated intravascular coagulation
DM:	diabetes mellitus
DNA:	deoxyribonucleic acid
EDTA:	ethylenediamine tetraacetic acid
ESICM:	the European Society of Intensive Care Medicine
FiO_2:	fraction of inspired oxygen

FSP:	fibrinogen split products
GCS:	Glasgow coma scale
GPI:	glycosyl-phosphatidylinositol
HIV:	human immunodeficiency virus
HR:	heart rate
HRP-Streptavidine:	Horseradish-Streptavidine
ICU:	Intensive care unit
IL-1:	interleukin-1
IL-6:	interleukin-6
INR:	international normalized ratio
K:	potassium
KDIGO:	Kidney Disease Improving Global Outcomes
LBP:	lipopolysaccharide binding protein
LODS:	Logistic organ dysfunction system
LPS:	lipopolysaccharide
LTA:	lipoteichoic acid
MAP:	mean arterial pressure
mCD14:	membrane bound CD14
MD2:	myeloid differentiation factor 2
MEDS:	Mortality in Emergency Department Sepsis
MODS:	Multiple Organ Dysfunction Score
MPM:	Mortality probability model
MRSA:	methicillin-resistant Staphylococcus aureus
Na:	sodium
NEMS:	Nine Equivalents of Nursing Manpower Use Score
NLRs:	Nucleotide-binding oligomerization domain-like receptors
NO:	nitric oxide
NYHA IV:	New York Heart Association Class IV
$PaCO_2$:	Partial pressure of carbon dioxide

PAF:	platelet activating factor
PAMPs:	Pathogen-associated molecular patterns
PaO_2:	partial pressure of oxygen
PCR:	polymerase chain reaction
PCT:	procalcitonin
PEEP:	positive end-expiratory pressure
PG:	peptidoglycan
PIRO:	Predisposition, Infection, Response, Organ dysfunction
PLT:	platelet
PRRs:	Pattern recognition receptors
PSEP:	presepsin
PT:	prothrombin time
PTT:	partial thromboplastin time
qSOFA:	quick SOFA
REMS:	Rapid Emergency Medicine Score
RLRs:	Retinoic acid-inducible gene-I-like receptors
RNA:	ribonucleic acid
ROC:	Receiver Operating Characteristic
RR:	respiratory rate
SAPS:	Simplified acute physiology score
SBP:	systolic blood pressure
SCCM:	the Society of Critical Care Medicine
sCD14:	soluble CD14
sCD14-ST:	soluble CD14 subtype
SD:	standard deviation
SIRS:	Systemic inflammatory response syndrome
SIS:	the Surgical Infection Society
SOAP:	Sepsis occurrence in acutely ill patients
SOFA:	Sequential (sepsis-related) Organ Failure Assessment

TF:	tissue factor
TFPI:	tissue factor pathway inhibitor
TISS:	Therapeutic Intervention Scoring System
TLRs:	Toll-like receptors
TMB:	tetramethylbenzidine
TNF*α*:	tumor necrosis factor-α
VAP:	ventilator-associated pneumonia
WBC:	white blood cell

1. INTRODUCTION AND BACKGROUND

Sepsis is a life-threatening condition, with poor and highly variable clinical manifestations, difficult to identify and diagnose.

In order to generate a unified definition of sepsis, three international consensus conferences on sepsis definitions, known as Sepsis-1, Sepsis-2, and Sepsis-3 Consensus Conferences, were held in 1991, 2002, and 2016, respectively, (1-3).

Sepsis can be caused by various microorganisms, and can arise from different sites of infection.

Until 1990s, Gram-negative organisms were the most common causative pathogens of sepsis. Starting from 2000, the percentage of Gram-positive bacteria is reported to be higher than the one of Gram-negative bacteria as causative organisms of sepsis. At present, very often, it has been reported that Gram-negative and Gram-positive bacteria in a similar percentage are causing sepsis (4).

Respiratory infection is reported to be the most common site from which sepsis arises (4-7), followed by abdominal and genitourinary infections. Less often, skin and soft tissues or intravascular devices can be the source of infection. In a number of cases the site of infection remains unknown.

Sepsis affects persons of all ages. The elderly and persons with chronic comorbidities are at a higher risk for developing sepsis. Some studies have reported that more than a half of septic patients have at least one underlying chronic disease (8).

Historically, it has been, and it has remained, a disease that causes millions of deaths per year worldwide, as well as a major therapeutic and economic concern.

The pathogenesis of sepsis is a complex process intermediated by host immune cell activation, production and release of inflammatory cytokines, resulting in activation of complement, coagulation and fibrinolytic system. Different cell receptors are implicated in pathogen recognition. After pathogen recognition, additional cells are activated, in order to eliminate the causative microorganism. The inflammatory response created as a result of host cell activation is mainly responsible for organ systems dysfunction, a hallmark of sepsis.

Sepsis is a response of host immune cells activation due to microbial agent invasion. In early 1900s, William Osler noted: “it appears that patients are dying not from their infections but rather their reaction to them”. Despite new knowledge in the pathogenesis of sepsis, there are still many unknown and unexplained mechanisms of development of organ dysfunction.

Given the importance of early diagnosis of sepsis and timely administration of appropriate antibiotic therapy, various biomarkers have been discovered to aid a rapid evaluation of critically ill patients. Among them, soluble CD14 subtype (sCD14-ST), also known as presepsin (9), is a promising biomarker for early diagnosis of sepsis (9).

As for many syndromes, there is still no golden standard diagnostic test for sepsis.

Although sepsis incidence is increasing and mortality rate is still very high (7, 10, 11), treatment recommendations from Sepsis-3 task force seem to improve the disease outcome.

1.1. Definition of sepsis

Sepsis [σηψις] is originally a Greek word, known for more than 2700 years, meaning “the decomposition of animal or vegetable organic matter in the presence of bacteria” (12).

In the nineteenth century, the knowledge about the origin and transmission of infectious diseases grew. Findings by Robert Koch (13)**,** Joseph Lister (14), and Louis Pasteur (15) led to the establishment of the ‘germ theory’.

For more than a century, pathogens were thought to be the sole causes of infection and sepsis.

By 1990, the role of innate immunity in response to infection was thought to be as important as the pathogen itself.

For better understanding of sepsis, a number of definitions have been developed with the aim to have a more unified point of reference when performing clinical trials. One of the first concepts was the definition of sepsis as “*a systemic host response to an infection*” (16).

In 1991, the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) held a Consensus Conference, with the aim of developing a set of definitions that could be applied in patients with sepsis (1). The goals of this consensus conference were to improve the early recognition of septic patients, and to enable standardization of terminology for

better use of information derived from different studies. The severity of sepsis was categorized based on hemodynamic disorders and organ dysfunction. The use of scoring systems for predicting disease outcome was suggested, and guidelines for treatment of septic patients were recommended. In 1992, ACCP/SCCM published the consensus definitions of sepsis and the term "*systemic inflammatory response syndrome–SIRS*" was introduced (1). SIRS was defined as a widespread inflammation that occurs in patients with different infectious and non-infectious conditions.

SIRS was described as a systemic inflammatory innate immune response regardless of cause, that comprises one or more of the following clinical manifestations: (1) a body temperature greater than 38°C or less than 36°C; (2) a heart rate greater than 90 beats per minute; (3) tachypnea manifested by respiratory rate greater than 20 breaths per minute, or hyperventilation, indicated by $PaCO_2$ of less than 32 mmHg; and (4) an alteration in the white blood cell count, such as a count greater than 12,000/mm³, a count less than 4,000/mm³, or the presence of more than 10 percent immature neutrophils ("bands") (Box-1) (1).

In 2001, a second consensus conference was held, sponsored by the Society of Critical Care

Medicine (SCCM), European Society of Intensive Care Medicine (ESICM), American College of Chest Physicians (ACCP), American Thoracic Society (ATS), and the Surgical Infection Society (SIS) (2), aiming to review and improve current definitions of sepsis. After revision, due to a lack of supporting evidence, there were no changes in the current definitions of sepsis, severe sepsis and septic shock. Concepts as described 10 years earlier remained unchanged and as such were used by clinicians and researchers. A broad list of signs and symptoms of sepsis was added to the existing definitions for better understanding of the clinical response to infection (Table 1) (2).

Box-1. SIRS (Systemic Inflammatory Response Syndrome)

1.*Temperature >38°C or <36°C*

2. Heart rate >90/min

3. Respiratory rate >20/min or $PaCO_2$ <32 mmHg (4.3 kPa)

4. White blood cell count >12,000/mm³ or <4,000/mm³

or >10% immature bands

SIRS remained a useful concept but diagnostic criteria for SIRS, published in 1992, were considered to be nonspecific. A hypothetical model for staging sepsis, named PIRO (*Predisposing*

factors, nature of *Infection*, host *Response*, degree of *Organ* dysfunction), was introduced. The model was based on premorbid conditions and predisposing factors, the nature of infection, host's response characteristics, and the degree of organ dysfunction.

Until 2016, definitions for *SIRS*, *sepsis*, *severe sepsis* and *septic shock* were as follows (2):

Systemic inflammatory response syndrome–SIRS *was defined as an inflammatory process, independent of its cause*. It could be seen in different infectious and non-infectious conditions. In absence of infection, SIRS was also present in patients with burns, hemorrhagic shock, multiple traumas and tissue injury, pancreatitis and other inflammatory conditions.

Sepsis was defined as *a clinical syndrome characterized by the presence of both infection and a systemic inflammatory response.*

Severe sepsis *was defined as a sepsis with organ dysfunction. Organ dysfunction was described using scoring systems such as the Sequential (Sepsis-related) Organ Failure Assessment score (SOFA score)* (17).

Septic shock *was defined as a state of acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes.*

The last revision of sepsis definition was done recently. In 2016, the third ESICM/SCCM consensus conference, known as Sepsis-3, was held with the aim to re-evaluate previous sepsis definitions and issue new sepsis definitions based on new pathobiology knowledge (Table 2) (3). ***Sepsis*** was defined as *a life-threatening organ dysfunction caused by a dysregulated host response to infection* (3). A definition of sepsis previously used by Czura was also endorsed by the task force: "*sepsis is a life-threatening condition that arises when the body's response to infection injures its own tissues and organs*" (18).

Although SIRS criteria were thought to still be helpful in recognizing sepsis, as well as the list of signs and laboratory findings, introduced in 2001, new criteria for sepsis recognition were set. A SOFA score ≥2 points was set as a criterion for identifying septic patients in intensive care units (ICUs). A new simpler score, which derived from the original SOFA score, was introduced to identify septic patients at bedside, named qSOFA (Box-2). qSOFA is a modified version of SOFA score that evaluates only three parameters: mentation, systolic blood pressure, and respiratory rate (Box-2) (3).

Table 1. Expanded list of signs, symptoms, and laboratory parameters of sepsis introduced in 2001 by SCCM/ESICM/ACCP/ATC/SIS task force as diagnostic criteria for sepsis

Infection	General parameters	Inflammatory parameters	hemodynamic parameters	organ dysfunction	tissue perfusion parameters
documented or suspected	Fever >38.8°	WBC count >12,000/µl	MAP<70 mmHg, or SPB<90 mmHg, or a decrease of SBP> 40 mmHg	PaO_2/FiO_2: <300	lactate level >3 mmol/l
	Fever <36°C	WBC count <4,000//µl	mixed oxygen saturation >70%	acute oliguria (urine output <0.5 ml/kg/h	decreased capillary refill, or mottling
	HR: >90 beats/min or >2 SD above the normal value for age	normal WBC count with >10% immature cells	cardiac index <3.5 l/min x m^2	creatinine increase ≥0.5 mg/dl	
	RR: >20 breaths/min	plasma CRP: > 2SD above the normal value		INR: >1.5 or aPTT: >60 s	
	altered mental status	plasma PCT: > 2SD above the normal value		Ileus	
	significant edema or positive fluid balance (<20ml/kg over 24 h)			PLT count < 100,000/µl	
	plasma glucose >110 mg/dl or >7.7mmol/l, in absence of diabetes			Total bilirubin > 4 mg/dl or 70 mmol/l	

Adapted from Levy et al. (2)

Abbreviations: HR-heart rate, RR-respiratory rate, SD-standard deviation; WBC-white blood cell; CRP-C-reactive protein, PCT-procalcitonin, MAP-mean arterial pressure, SPB-systolic blood pressure, PaO_2-partial pressure of oxygen, FiO_2-fraction of inspired oxygen; INR-international normalized ratio, aPTT-activated partial thromboplastin time, PLT-platelet

Each component was assigned by one point. A qSOFA score ≥ 2 points indicates organ dysfunction, and was set as a clinical criterion for recognizing septic patients outside the ICUs.

Box-2. Quick Sequential Organ Failure Assessment score-qSOFA score parameters

Respiratory rate ≥22/min

Change in mental status, GCS<13 points

Systolic blood pressure ≤100 mmHg

Abbreviations: GCS-Glasgow coma scale

According to new Sepsis-3 definitions, ***septic shock*** *was defined as a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality* (3).

As clinical criteria for supporting definition were set: 1) the need for vasopressor therapy to maintain MAP ≥65 mmHg; and 2) serum lactate level >2 mmol/L persisting after adequate fluid resuscitation.

Based on findings that a great number of patients in ICUs fulfil the SIRS criteria and never develop sepsis, the term SIRS was abandoned.

The 2016 ESICM/SCCM task force compared SIRS criteria to scoring systems: Logistic Organ Dysfunction System (LODS) and Sequential Organ Failure Assessment (SOFA). Based on the statistical analysis, SOFA and LODS showed a similar predictive capacity; however, SOFA score has been designed to evaluate organ dysfunction or organ failure in septic patients (19, 20)**,** and as such was recommended for evaluation of organ dysfunction in septic patients. The total SOFA score of 2 points or more represents organ dysfunction.

New Sepsis-3 terms and definitions are listed below (3).

New Sepsis-3 terms and definitions

- Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection.

- Organ dysfunction is defined as an acute change in total SOFA score ≥2 points consequent to the infection

- A SOFA score ≥2 reflects overall mortality risk of approximately 10% in a general population with suspected infection.

- Sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs.

- qSOFA can be used for prompt identification of ICU patients with suspected infection

- Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.

- Patients with septic shock can be identified as sepsis patients with persisting hypotension requiring vasopressors to maintain MAP≥65 mmHg and having serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation. Hospital mortality exceeds 40% in patients who fulfils these criteria.

1.2. Scoring systems

To predict the disease outcome, and to assess the degree of organ dysfunction and severity of disease in critically ill patients, different scoring systems can be used.

There is a large number of scoring systems that are widely used in critically ill patients. The combined use of scores provides more accurate indications of severity of disease and prognosis.

Vincent and Moreno (21), in a clinical review, divided scores that can be used in all ICU patients into three classes: 1) scores that assess disease severity on admission and can be used to predict

disease outcome such as Acute Physiology And Chronic Health Evaluation (APACHE), Simplified Acute Physiology Score (SAPS), Mortality Probability Model (MPM), etc.; 2) scores that evaluate the presence and severity of organ dysfunction, such as Multiple Organ Dysfunction Score (MODS), Sequential Organ Failure Assessment (SOFA), etc.; and 3) scores that assess nursing workload use, such as Therapeutic Intervention Scoring System (TISS), Nine Equivalents of Nursing Manpower Use Score (NEMS), etc.

In the present study, we used a severity of disease and outcome prediction score: Acute Physiology And Chronic Health Evaluation score (APACHE II) and an organ dysfunction score: Sequential (sepsis-related) Organ Failure Assessment (SOFA), to evaluate disease severity and disease outcome in our septic patients, as well as to explore the association of these scores with presepsin concentration.

Both scores, APACHE II and SOFA, are widely used in clinical trials on septic patients.

1.2.1. Acute physiology and chronic health evaluation II score – APACHE II score

In 1978, the original Acute Physiology And Chronic Health Evaluation (APACHE) score was developed, in order to classify ICU treated patients according to severity of illness and to evaluate their care (22). It was composed of two parts: acute physiology score (APS)–for assessing the degree of acute illness; and a preadmission evaluation of patient's chronic health status. To calculate the score, 34 physiologic variables had to be measured and patient's chronic health status had to be assessed.

In 1985, to simplify the use of APACHE score, the original model was revised and, as a result, the APACHE II score was developed (23). The revised APACHE II score (Table 2), consists of 12 physiological variables listed below:

1) vital signs: heart rate, mean arterial pressure, respiratory rate, temperature and Glasgow coma scale (GSC);
2) variables obtained from routine blood tests: hematocrit, white cell count, serum bb sodium, serum potassium, serum creatinine; and
3) two variables obtained from arterial blood tests: pH and $PaCO_2$.

The APACHE II score also includes measures for patient's age, severe chronic disease (heart failure class IV, cirrhosis, chronic lung disease or hemodialysis) and surgical status of the patient.

For each physiologic variable, the worst value recorded in the first 24 hours is used. A total maximum score of 71 points can be obtained. The highest the obtained total maximum score, the worst the outcome.
To improve the accuracy for predicting hospital mortality, APACHE III score was developed in 1991, and revised and validated in 1998. In 2006, Zimmerman et al. developed APACHE IV score.

APACHE II score is one of the most commonly used scores for mortality prediction and severity of disease classification in critically ill patients, widely incorporated into clinical trial design.
Badrinath et al. (19), in a recently published study, compared several scoring systems in septic patients. They calculated APACHE II score, Rapid Emergency Medicine Score (REMS), Sequential Organ Failure Assessment (SOFA), Multiple Organ Dysfunction Score (MODS), Predisposition, Infection, Response and Organ dysfunction (PIRO), and Mortality in Emergency Department Sepsis (MEDS) on 193 septic patients. All scores were significantly higher in non-survivors compared to survivors. They found APACHE II score to be the best score for disease stratification and outcome prediction. The APACHE II score showed the best discriminative power and sensitivity compared to other calculated scores.

The second part of APACHE II score consists of points assigned according to patient's age:

≤44 years	0 points
45-54 years	2 points
55-64 years	3 points
65-74 years	5 points
≥75 years	6 points

The third part of APACHE II score consists of chronic health points. If a patient has a history of severe organ system insufficiency or is immunocompromised, points are assigned as follows:

1) non-operative or emergency postoperative patients: 5 points
2) elective postoperative patients: 2 points

Table 2. The acute physiology and chronic health evaluation score (APACHE II)

	High abnormal range					Low abnormal range			
Physiologic variable	4	3	2	1	0	1	2	3	4
Rectal temperature (°C)	>41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤29.9
MAP (mmHg)	≥160	130-159	110-129		70-109		50-69		≤49
Heart rate	≥180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory rate (non-ventilated/ventilated)	≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygenation: A-aDO_2, or PaO_2 (mmHg)									
1. FiO2>0.5, record A-aDO_2	≥500	350-499	200-349		<200				
2. FiO_2<0.5, record only PaO_2					PaO2>70	PaO2 61-70		PaO2 55-60	PaO2<55
Arterial Ph	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
Serum sodium (mmol/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
Serum potassium (mmol/L)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
Serum creatinine (mg/100ml) (double point score for acute renal failure)	≥3.5	2-3.4	1.5-1.9		0.6-1.4		<0.6		
Hematocrit (%)	≥60		50-59.9	46-49.9	30-45.9		20-29.9		<20
WBC count (total/mm^3)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
GCS (score=15-actual)									
Total acute physiology score (APS)									
Serum HCO_3 (venous/mmol/L), use if no ABGs	≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	<15

Abbreviations: MAP-mean arterial pressure; *A-aDO_2*-alveolar-arterial difference in oxygen tension; *PaO_2*-partial arterial pressure of oxygen; *FiO_2*-fraction of inspired oxygen; WBC-white blood cell; GCS-Glasgow coma scale; Adapted from Wagner et al., 1984 (24)

Organ insufficiency or immunocompromised state must be evident prior to hospitalization and conform to the following criteria (24):

1) Liver biopsy proven cirrhosis and documented portal hypertension, episodes of past upper gastro-intestinal bleeding or prior episodes of hepatic encephalopathy/coma;
2) Heart insufficiency: New York Heart Association Class IV (NYHA IV);
3) Respiratory insufficiency: chronic restrictive, obstructive or vascular disease resulting in severe exercise restriction, i.e. unable to climb stairs or perform household duties, or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40 mmHg), or respirator dependency;
4) Renal insufficiency: chronic dialysis;

5) Immunocompromised state: patient has received immunosuppressive therapy (chemotherapy, radiation, long term or recent high dose steroids), or has a disease that is sufficiently advanced to suppress resistance to infection, e.g. leukemia, lymphoma, AIDS.

Total APACHE II score is equal to the sum of Acute Physiology score + age points + chronic health points.

1.2.2. Sequential (sepsis-related) Organ Failure Assessment –SOFA score

In 1994, the European Society of Intensive Care Medicine (ESICM) convened a consensus meeting to create a score that would as much as possible reflect the degree of organ dysfunction or failure. They named the new score “sepsis-related organ failure assessment” (SOFA) (20). Even though two similar scores existed at that time: the “Multiple Organ Dysfunction Score” (MODS) designed by Marshal et al., and the “Brussels Score” designed by Bernard et al., in both scores the cardiovascular component of the score was difficult to calculate. The ESICM meeting participants tried to create a simpler score, in which all variables would be measurable and scaled.

The SOFA score (Table 3) assesses the function of six organ systems: respiratory, cardiovascular, renal, hepatic, central nervous system, and coagulation. The incorporated systems were selected based on literature review. The function of each system was scored from 0 points (normal function) to 4 points (most abnormal function), giving a total possible score of 0 to 24 points (20). For each variable the worst value on each day had to be recorded.

The SOFA score is the most commonly used clinical score to evaluate development of organ dysfunction during sepsis.

Although SOFA score was not designed to predict outcome, but severity of disease, since the degree of organ dysfunction is related to mortality, a relation between mortality and organ dysfunctions exists, and different studies have found such a relation. The higher the severity and number of organ dysfunction, the greater the risk of developing septic shock and thus the worst is the outcome of disease.

Table 3. The SOFA score

SOFA score	1	2	3	4
Respiration				
PaO_2/FiO_2, *mmHg*	<400	<300	<200/ respiratory support	<100/ respiratory support
Coagulation				
platelets x $10^3/mm^3$	<150	<100	<50	<20
Liver				
Bilirubin mg/dl or	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
(µmol/l)	20-32	33-101	102-204	>204
Cardiovascular				
Hypotension	MAP<70 mmHg	dopamine ≤5 or dobutamine any dose	dopamine >5 or epinephrine≤0.1 or norepinephrine ≤0.1	dopamine>15 or epinephrine>0.1 or norepinephrine>0.1
CNS				
Glasgow coma scale	13-14	10 – 12	6 - 9	<6
Renal				
Creatinine mg/dl	1.2-1.9	2.0-3.4	3.5-4.9	>5.0
(µmol/l) or	110-170	171-299	300-440	>440
urine output			or <500 ml/day	or <200 ml/day

Abbreviations: PaO_2- partial oxygen pressure, FiO_2- fraction of inspired oxygen, CNS- central nervous system, MAP- mean arterial pressure. Vasoactive agents administered for at least 1 hour (dose expressed in µg/kg/min)
Adapted from Vincent et al., 1996 (20).

Shortly after the introduction of SOFA score as a score designed to evaluate organ dysfunction in septic patients, studies have shown its correlation with disease outcome. Moreno et al. (25), in a prospective multicenter multinational study, evaluated the SOFA score on 1,449 patients admitted to forty ICUs from Europe, Australia, South and North America. SOFA score was calculated on admission and every 24 hours until discharge. They calculated total maximum SOFA score, defined as the sum of worst scores for each component of the SOFA score, and delta SOFA score defined as total maximum SOFA score minus SOFA score on admission. They found a good correlation of total maximum SOFA score with disease outcome. The delta SOFA, defined as the degree of organ/dysfunction found after ICU admission, also showed a good correlation with disease outcome.

The prognostic capacity of the SOFA score was also evaluated in other studies. In a recent retrospective cohort study on 184,875 critically ill patients admitted to Australian and New Zealand ICUs, Raith et al. (26) evaluated the prognostic accuracy of SOFA score, SIRS criteria, and

qSOFA in patients with infection. They found that sepsis defined as SOFA score of 2 points or greater showed a better prognostic accuracy for in-hospital mortality compared to the SIRS criteria or the qSOFA equal to or greater than 2 points.

1.3. Epidemiology and etiology of sepsis

Sepsis etiology and epidemiology have changed over decades. Even though epidemiological studies have reported a decreasing trend of mortality from sepsis, its incidence has increased. Studies have also reported a switch in sepsis causing pathogens from Gram-negative mostly recorded in the 1980s to Gram-positive organisms being at present the most common causative agents of sepsis, as well as an increasing fungal etiology of sepsis. More frequently isolated Gram-positive bacteria in septic patients over the last years may be due to more frequent use of invasive diagnostic and therapeutic procedures as well as the wide use of antibiotics, thus creating conditions for generation of resistant strains.

1.3.1. Incidence and mortality from sepsis

Until 2000, sepsis was among the ten leading causes of death. Recently published reports by the Division of Vital Statistics reported sepsis to be among the fifteen leading causes of death in the United States (27).

In Kosovo, there are no published data on sepsis epidemiology.

In Croatia, the first data for incidence and mortality from sepsis were published in 2006. Gašparović et al. (28), in the pilot phase of a national comparative clinical database project, running over a one-year period, reported sepsis and severe sepsis to be the third most common reason for ICU admission; representing 8.6% of all admissions to the selected ICUs.

They reported the overall mortality from sepsis syndrome, severe sepsis and septic shock in Croatian ICUs to be 29%, 35%, and 34%, respectively. Degoricija et al. (29), in a retro-prospective six-year study on sepsis epidemiology in medical ICUs in Croatia, reported that sepsis admissions accounted for 6.3% of all ICU admissions during the study period. They reported mortality rate for

sepsis, severe sepsis, septic shock, and multiple organ dysfunction syndrome (MODS), to be 17.0%, 33.7%, 72.1%, and 74.4%, respectively.

In 2005, Zahorec et al. (5), in a prospective epidemiological study on 124 patients with severe sepsis and septic shock, treated in ICUs of the Republic of Slovakia, reported the incidence and overall hospital mortality to be 7.9% and 51.2%, respectively.

Beović et al. (6), in a study of severe sepsis treated in 95.6% of all Slovenian ICUs, reported the estimated incidence of severe sepsis in ICUs to be 1.18 cases per 1,000 inhabitants/year. A 28-day mortality was reported to be 45.1%.

In the European Sepsis Occurrence in Acutely ill Patients (SOAP) epidemiological multicenter observational study on sepsis in ICUs, Vincent et al. (4)**,** on a total of 1,177 patients with sepsis, reported differences among European ICUs in mortality and incidence of sepsis. The rate of sepsis was reported as low as 18% in Switzerland and up to 73% in Portugal. The rate of severe sepsis was reported to be 10% in Switzerland, and up to 64% in Portugal. The lowest ICU mortality from sepsis was reported in Switzerland, and the highest in Italy, with 10% and 35%, respectively. The ICU mortality of patients with severe sepsis and septic shock was reported to be 32.2% and 54.1%, respectively. Hospital mortality from sepsis was reported to be 20% in Germany, and up to 47% in the Netherlands.

Angus et al. (7), in an observational study on sepsis epidemiology, reported the national incidence rate of severe sepsis in the United States to be 3.0 cases per 1,000 population, and overall hospital mortality rate of 28.6%. Martin et al. (10) reported an increase of the incidence of sepsis over a 22-year period, from 82.7 cases per 100,000 population to 240.4 cases per 100,000 population, with an annual increase of 8.7%.

The global number of sepsis cases is estimated to be more than 31 million, leading to 5 million deaths per year (11). In a literature review from 2015, Fleischmann et al. (11) reported global hospital mortality from sepsis and severe sepsis to be 17% and 26%, respectively.

All recent studies have reported an increasing incidence and a decreasing mortality from sepsis and septic shock, although the overall number of deaths is still very high due to the higher incidence.

1.3.2. Demographic features of patients with sepsis

There is a slight difference between genders in the overall risk for developing sepsis, with a predominance of male gender in most published studies (4-8, 28, 29), although the reasons are unclear.

Sepsis is more common in the elderly. In most studies, more than half of patients with sepsis were reported to be older than 65 years. Vincent et al. (4) reported the median age of septic patients to be 64 years. Similarly, Angus et al. (7) found the mean age of patients with severe sepsis to be 63.8 years, with approximately 60% of patients ≥65 years. Patients with comorbid conditions are more likely to develop sepsis. Epidemiological studies have reported that more than half of patients with sepsis have at least one accompanying comorbidity.

At a greater risk for developing sepsis are the elderly >65 years, patients with diabetes, those who are on immunosuppressive therapy (immunosuppressants, chemotherapy, long term corticosteroid therapy), patients with chronic renal or liver disease, those with chronic obstructive pulmonary disease, patients with cancer, those with indwelling intravascular catheters or lines, patients who recently had surgery or other invasive procedures, patients with burns or skin infections, and intravenous drug abusers. At a greater risk for developing sepsis are also pregnant women or women who had given birth or had a miscarriage.

In the European EPISEPSIS study, Brun-Buisson et al. (8) found that more than half of patients with severe sepsis have at least one chronic organ system dysfunction.

Higher mortality rates have been reported among patients with underlying comorbidities (30). In most epidemiological studies, the association of the number of organs failing during sepsis with mortality rate has been evaluated. The mortality rate increases with each failing organ (30). The greater the number of organs failing during sepsis the higher the mortality (4-7, 10, 29, 30).

1.3.3. Etiology of sepsis

Sepsis can arise from different sites of infection. In general, respiratory infections are more commonly complicated by sepsis. The lung has been found to be the most common site of infection (4-7, 30), followed by abdominal and genitourinary infections. Other possible sites of

infections are: the skin and soft tissue, intravascular devices, central nervous system, etc. In approximately 15% of patients, the site of infection remains unknown (30).

For many decades, sepsis was thought to be a disease caused exclusively by bacteria.

Recent studies has revealed that there has been a shift of microorganisms causing sepsis, from the predominant Gram-negative bacteria until late 1980s, to Gram-positive bacteria at present. Martin et al. (10), in a large retrospective 22-year epidemiological study, reported Gram-negative bacteria to be the most common isolates in septic patients until 1987; in each subsequent year until 2000, Gram-positive bacteria were reported to be the predominant causative organisms isolated from blood in patients with sepsis, accounting for more than half of all isolates. The same study reported Gram-negative bacteria and fungi as microorganisms causing sepsis in 37.6% and 4.6%, respectively.

Studies have reported an increasing incidence of fungal sepsis over the last decade, as well as an increasing incidence of polymicrobial sepsis. In 2006, Vincent et al. (4), in a large prospective European multicenter study, reported Gram-positive bacteria to be isolated from 40% of septic patients treated in ICUs; Gram-negative bacteria and fungi accounted for 37% and 17%, respectively.

Among Gram-positive cocci, most commonly reported pathogens are: *Staphylococcus aureus*, (31) and coagulase negative *Staphylococcus* (29, 83). Methicillin-resistant *Staphylococcus aureus* is an important pathogen responsible for intra-hospital sepsis. Among Gram-negative bacilli, *Escherichia coli* remains the most commonly isolated pathogen (29, 83).

Fungal etiology of sepsis has increased rapidly. *Candida albicans* has been most frequently reported as a pathogen causing fungal sepsis (10, 31, 83). At present, other Candida species are reported to cause nosocomial sepsis (32).

1.4. Pathogenesis and pathophysiology of sepsis

The pathogenesis of sepsis is a complex process initiated by the invasion of pathogens in the bloodstream from different foci in which a deficient local immunological response did not result in pathogen elimination, and as such has allowed the establishment of sepsis.

1.4.1. Immunopathogenesis of sepsis

The first responder to the invasion of microorganisms is the innate immune system.

As a result of phagocytosis and bacterial degradation, structural components of bacteria are released into circulation and then recognized by receptors on the surface of immune cells, known as pattern recognition receptors (PRRs) (33). PRRs are widely spread on the surface of innate immune cells, such as macrophages, dendritic cells, fibroblasts, epithelial and endothelial cells, and they play a central role in recognition of structural bacterial components and consequently in initiation of host immune response. They recognize two types of molecules: danger signals derived from pathogens, the so-called Pathogen-Associated Molecular Patterns (PAMPs), and components released from host cells during cell damage, the so-called Danger-Associated Molecular Patterns (DAMPs).

To date, several classes of pattern recognition receptors have been identified (34): 1) Toll-like receptors (TLRs); 2) Retinoic acid-inducible gene-I-like receptors or RIG-I-like receptors (RLRs); 3) Nucleotide-binding oligomerization domain-like receptors or NOD-like receptors (NLRs); and 4) DNA receptors (cytosolic sensors for DNA). Pattern recognition receptors recognize various PAMPs, such as lipopolysaccharide (LPS), peptidoglycan (PG), lipoteichoic acid (LTA), lipoproteins, fungal glycan, bacterial or viral DNA or RNA (34, 35).

The most widely studied pattern recognition receptors are Toll-like receptors.

In humans, ten Toll-like receptors (TLRs) have been identified (36). Toll-like receptors can be expressed on the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) or within intracellular compartments (TLR3, TLR7, TLR8, and TLR9). Different TLRs sense different pathogen components. TLR2 senses peptidoglycan and lipoteichoic acid of Gram-positive bacteria and other bacterial and fungal components, whereas TLR4 recognizes lipopolysaccharide (LPS) of Gram-negative bacteria, as well as other bacterial, viral, and fungal components. TLR4 is also implicated in recognition of lipoteichoic acid of Gram-positive bacteria (36).

TLRs are considered to be the primary sensors of pathogens.

The biologically active component of the wall of the Gram-negative bacteria is lipopolysaccharide (LPS), also known as endotoxin. In Gram-positive bacteria there is no endotoxin but their cell wall is built of peptidoglycan and lipoteichoic acid that account for biologically active components.

In sepsis animal models, the pathogenesis of Gram-negative bacterial sepsis and the interaction between endotoxin and host immune cells has been studied mostly. After being released into circulation, LPS binds to a serum protein, known as lipopolysaccharide binding protein (LBP). As a complex molecule, LPS/LBP is transported to host cell membrane. LBP transfers LPS to another receptor on the cell surface membrane, the so-called cluster of differentiation 14 (CD14), a glycosyl-phosphatidylinositol-linked (GPI-linked) cell surface protein (37). Although, when identified, CD14 was originally thought to be a specific co-receptor that mediates activation of monocytes by LPS, at present it is known that CD14 also participates in the activation of host immune cells by Gram-positive bacteria cell wall components such as peptidoglycan (38). There are two forms of CD14: membrane bound CD14 (mCD14) and soluble CD14 (sCD14). Dendritic cells, fibroblasts, smooth muscle cells and vascular endothelium, that are CD14 negative, are able to respond to LPS through the soluble form of CD14 (sCD14) (39). Despite the discovery of CD14, it remained unclear how the immune cells are activated after the creation of LPS-LBP complex, when it was known that mCD14 had no intracellular tail (39)**.** The discovery of Toll-like receptor family (TLRs) brought clarity to this matter. A small protein known as myeloid differentiation factor 2 (MD2), associated with the extracellular region of TLR4, is another component of the LPS-recognition. The LPS/LBP complex after binding to CD14, mediated by MD2, finally binds to TLR4 (36).

The activation of TLR4 results in release of cytokines into the bloodstream from host immune cells.

As a response to an infection, host cells release into circulation a large number of inflammatory mediators. Sepsis is a condition in which the host pro- and anti-inflammatory innate immune response is initiated at the same time, as a reaction to the presence of microbial agents (40, 41). The balance between the pro- and anti-inflammatory responses determines the outcome of the disease (40). Sepsis is a complex clinical syndrome that results from a harmful or damaging host response to infection. Sepsis develops when the initial, appropriate host response to an infection becomes amplified and then dysregulated (39).

The initial interaction between the host and the microorganism leads to the activation of innate immune response, the aim of which is to localize and prevent the spread of the infection and repair the tissue damage. This response consists of phagocytes and endothelial cells activation. As a result, production of both pro-inflammatory and anti-inflammatory mediators is initiated, in order to maintain an immunological balance.

Two decades ago, the term systemic inflammatory response syndrome (SIRS) was introduced to describe the pro-inflammatory innate immune response of host cells to the presence of an invading microbial agent. The most potent pro-inflammatory cytokines that induce SIRS are considered to be tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6), capable to activate target immune cells and induce the production of more inflammatory cytokines. SIRS is thought to be followed by compensatory anti-inflammatory response syndrome (CARS) (35), characterized by an enormous release of anti-inflammatory cytokines. Later on, studies have shown that the pro- and anti-inflammatory response occurs at the same time and that SIRS and CARS are not two different phases of the same septic process.

1.4.2. Coagulation disorders in sepsis

Coagulation disorders and inflammation are closely linked, each activating the other (42).

Coagulation disorders in sepsis are a result of a procoagulant state and impaired function of anticoagulants. The procoagulant state is established as a result of interaction between tissue factor and inflammatory cytokines (39, 42). In normal conditions, coagulation and fibrinolysis are kept in balance, so blood freely flows in the vasculature without manifestations of bleeding or clotting. During sepsis, tissue factor is synthesized and expressed by the endothelium.

Disturbances in coagulation during sepsis start with activation of tissue factor (TF) (39, 42). When mononuclear and endothelial cells are stimulated by LPS or by pro-inflammatory cytokines, such as TNF-α, the tissue factor is expressed on their surface. TF binds and activates factor VII on the cell surface, thus creating the complex tissue factor/activated factor VII (TF/VIIa). The created complex TF/VIIa initiates the activation of factor X (Xa), leading to conversion of prothrombin into thrombin and fibrin clot formation (42, 43). The created complex TF/VIIa activates a protein with anticoagulant features, tissue factor pathway inhibitor (TFPI). During sepsis, TFPI is quickly consumed due to its limited amount in plasma (42). Another powerful anticoagulant with anti-inflammatory properties is protein C. Its impaired synthesis and quick consumption lead to the procoagulant state in sepsis (39, 42, 43).

In an inflammatory state, such as sepsis, platelet activating factor (PAF) is produced and then released into the bloodstream from activated host immune cells (endothelial cells, platelets,

neutrophils, monocytes, and macrophages). PAF initiates platelet adhesion to endothelium and leukocytes (42), thus platelets act as a surface for thrombin generation (44).

The coagulation disorders in septic patients may vary from light activation of the coagulation cascade to fulminant disseminated intravascular coagulation (DIC), which can be seen in patients with septic shock.

1.4.3. Pathophysiology of sepsis

Initial local infection is spread via blood stream to distant tissues and organs when the microorganisms overcome the host's immunological defense system. The presence of microorganisms in sterile host's sites triggers the production and the release of cytokines from host immune cells. Host immune cell activation and cytokine secretion lead to circulatory disorders, which are a result of the combination of intravascular volume depletion, vasodilatation, vascular leakage, and reduced cardiac output (45).

Altered endothelial function during inflammatory state such as sepsis is characterized by loss of barrier function, increased permeability, increased leukocyte adhesion, and coagulation disorders (43). Endothelial injury plays a central role in the pathogenesis of sepsis and organ dysfunction. It is common in all affected organs in sepsis, and leads to edema. The most common characteristic of cardiovascular dysfunction in septic patients is arterial hypotension. Arterial hypotension occurs due to hypovolemia, reduced vascular tone, and decreased myocardial function (46). Hypovolemia causes decreased heart filling and reduced ejection fraction (cardiac output), thus leading to imbalance in oxygen supply/demand in different organs. Endothelial cell activation leads to nitric oxide (NO) production. Nitric oxide and cytokines released from activated immune cells are responsible for myocardial depression. Nitric oxide is also responsible for impaired mitochondrial respiration. At present, it is known that mitochondria play an important role in sepsis-induced organ dysfunction (45).

Increased permeability of lung capillaries leads to interstitial edema, resulting in discrepancy between perfusion and ventilation, and arterial hypoxemia, clinically manifested as hyperventilation, thus leading to acute respiratory distress syndrome (ARDS) (47)**.** To reduce oxygen consumption by respiratory muscles, mechanical ventilation is recommended.

A common finding in sepsis is acute kidney injury (AKI), clinically presented as decreased urine output, increased serum creatinine levels, and may require renal replacement therapy. It can be attributed to hypovolemia and decreased renal perfusion, tubular necrosis or to cytokine and immune mediated microvascular and tubular dysfunction (48).

Jaundice is a clinical manifestation of cholestasis. In septic patients, it is related to impaired bile acids transport in hepatocytes. Bacteria, their toxins and cytokines can be responsible for liver dysfunction. Altered liver function in septic patients is a result of ischemia and consequent hypoxia, due to vasodilatation and hypoperfusion. (49). Hepatic failure is rare in septic patients. Liver injury is related to sepsis outcome, and hypoxic hepatitis is an unfavorable prognostic factor.

The nervous system is often affected during sepsis. Sepsis-related encephalopathy is a clinical manifestation that varies from mild confusion and disorientation to profound coma. Blood-brain barrier function is impaired due to increased systemic endothelial permeability, thus allowing cytokines and cells to enter the brain and cause perivascular edema (50).

Hematological and coagulation disorders during sepsis include decreased platelet count and disseminated intravascular coagulation (DIC). During sepsis, DIC can be identified by laboratory tests: prolonged activated partial thromboplastin time (aPTT), prolonged prothrombin time (PT), decreased platelet count, elevated fibrinogen split products (FSP), and elevated D-dimer (51).

The pathogenesis of organ dysfunction during sepsis is multifactorial and not entirely known. However, tissue hypoxia as a result of hypoperfusion is thought to be the major influencing factor. The failure of one organ leads to another organ dysfunction. The greater the number of organs with altered function, the worst the outcome of sepsis.

1.5. Diagnosis of sepsis

Early diagnosis and appropriate treatment of sepsis is crucial for better outcome of the disease. Signs and symptoms of sepsis are non-specific, thus making the early diagnosis of sepsis a real challenge for clinicians.

The combination of physical examination, history of disease, and laboratory parameters may be helpful for establishing diagnosis when sepsis is suspected.

Fever, increased heart rate, increased respiratory rate, and hypotension are signs and symptoms that can be seen not only in septic patients but in a large number of other inflammatory states. When combined with routine laboratory findings, such as increased white blood cell count, decreased platelet count, elevated serum creatinine levels, and elevated bilirubin levels, they can be helpful in diagnosing sepsis.

Blood cultures have remained a gold standard for the diagnosis of sepsis. When waiting for blood culture results, the diagnosis of sepsis may be delayed. So, in order to facilitate rapid diagnosis of sepsis, different sepsis biomarkers have been introduced lately. Although the sensitivity and specificity of sepsis biomarkers are highly variable, they are widely used in clinical trials for sepsis diagnosis.

Still, for an etiological diagnosis of sepsis and appropriate antimicrobial treatment, blood or/and site cultures are an irreplaceable tool. Identification of the causative pathogen is more likely when routine microbiological cultures are obtained before initiating antimicrobial therapy. At least two blood cultures should be drawn (aerobic and anaerobic) before starting the antimicrobial treatment. After the first dose of antimicrobial agent, sterilization of cultures can occur within minutes to hours (52). Microbiological cultures should be obtained from all suspected sites of infection: urine, respiratory secretions, cerebrospinal fluid, wound swab and culture, and other body fluids. In patients with intravascular catheters, if there are signs of infection at the catheter site, if the catheter was placed for more than 48 hours, or if the source of infection is unknown, a blood sample for culture should be drawn from the catheter, and another one from peripheral vein. (52). If there are signs of infection at the catheter site, the catheter should be removed, and swabs from the infected catheter should be sent for microbiological culture.

All samples for microbiological culture should be obtained before initiating antimicrobial treatment, if patients' clinical state allows to postpone antibiotic administration.

When possible, imaging techniques should be done early in order to confirm the potential source of infection, or identify a source of infection that requires surgical intervention.

A detailed patients' health history is important to provide information about risk factors for infection.

Blood culture results are available after 2 to 3 days. The positivity of blood culture results is related to prior treatment with antimicrobials, as well as to the amount of blood sample drawn for culture.

The use of blood cultures in diagnosing sepsis has its advantages in identifying microbial susceptibility, deescalating antimicrobial treatment or administering an appropriate antimicrobial. Still, it has its limitations. A large amount of blood is needed for increasing the possibility of identifying the microbial agent, and results are delayed for 2-3 days; as such, they are not helpful for initial decision and selection of antimicrobial treatment.

Molecular methods for microorganism detection, such as polymerase chain reaction (PCR), real-time PCR and microarray, are currently being used for sepsis prompt diagnosis. However, there is still not enough clinical experience for replacing the blood culture methods with these molecular non-culture techniques.

At present, rapid diagnosis of sepsis is based on clinical and laboratory findings as well as on sepsis biomarkers values.

1.5.1. Sepsis biomarkers

The importance of early and rapid diagnosis of sepsis, and timely administration of appropriate antibiotic therapy, has led to the need of discovering different sepsis biomarkers. Taking in consideration that standard microbiological cultures are often negative, and results are available after 2-3 days, the use of different sepsis biomarkers has facilitated early identification of patients who are at a greater risk for developing sepsis and septic shock. More than 170 biomarkers for diagnosing sepsis are reported in the literature. The US agency National Institutes of Health defines a biomarker as “a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathological processes or pharmacological response to a therapeutic intervention” (53).

Biomarkers can be used for diagnosis and prognosis of sepsis. Some of them can be used for disease stratification and mortality prediction. Other potential use of sepsis biomarkers is in antibiotic therapy guidance.

Marshall et al. (54) described four functional classes of biomarkers: *diagnostic biomarkers* serve to confirm the presence or absence of a disease; *monitoring biomarkers* serve as indicators of effectiveness of therapy; *surrogate biomarkers* are assigned to predict clinical outcome, and they need to be influenced by given therapy; *stratification (staging) biomarkers* are intended to serve for disease stratification based on outcome or severity.

Biomarkers that are most widely used for diagnosing sepsis are C-reactive protein (CRP) and procalcitonin (PCT). Another biomarker, frequently used over the last decade, is soluble CD14 subtype (sCD14-ST), known as presepsin. Several studies have found that a combination of biomarkers is more effective for differentiating infectious from non-infectious inflammatory response. The use of a single biomarker is not enough to undoubtedly determine whether the patient has or does not have sepsis.

The capacity of procalcitonin for diagnosing sepsis has been evaluated in clinical trials for more than two decades. However, the ability of procalcitonin to distinguish sepsis from non-sepsis inflammatory conditions is widely debated. Presepsin is a sepsis biomarker introduced in the last decade, and is still in an experimental phase of evaluation of its diagnostic ability. First results from different studies are promising–presepsin is referred to as an early sepsis biomarker with good capacity to distinguish sepsis from non-sepsis conditions.

1.5.1.1. C-reactive protein (CRP) and procalcitonin (PCT)

C-reactive protein was first described in 1930 by Tillet and Francis. Originally, it was named Fraction C**,** and was considered as a constituent of pneumococcus cells (55). It was first isolated from the sera of patients with lobar pneumonia, and was named according to its reactivity with C fraction of the wall of pneumococcus. It is a polypeptide belonging to the pentraxin family, built up of five subunits, each consisting of 206 amino acids (56). CRP is an acute phase reactant synthesized in hepatocytes, under the action of pro-inflammatory cytokines. CRP levels are associated with the presence of stimulus. Biological half-time of CRP is 19 hours. Serum levels of C-reactive protein depend on the size of its production, and as such, they reflect disease activity.

Use of C-reactive protein measurements for diagnosing bacterial infections, and distinguishing bacterial from viral or non-infectious conditions, has been rejected by some authors and approved by others. CRP reaches high levels in infected patients, but also in non-infectious inflammatory conditions, such as myocardial infarction, burns, surgery, rheumatic diseases, and a large number of other inflammatory conditions.

Its response is stronger in acutely ill patients; as a patient recovers the levels of CRP decrease. Its incapability to distinguish bacterial infections from non-infectious inflammatory conditions limits the diagnostic value of C-reactive protein if used as a single diagnostic biomarker.

Procalcitonin (PCT) is a precursor of calcitonin, a hormone that regulates calcium levels. PCT is a protein built of 116 amino acids, produced by C-cells of the thyroid gland. In healthy individuals it is secreted from thyroid tissue, and is present in blood at low concentrations (<0.1 ng/mL).

PCT was first described in 1993 by Assicot et al. (57), as a marker of bacterial infection in children with sepsis and septic shock. It was reported then that procalcitonin levels correlate with the severity of disease and decrease with patients' recovery.

During sepsis, procalcitonin is produced by non-thyroid tissues, including the liver, lung, pancreas, spleen, kidney, colon, and adipose tissues. In 2008, the American College of Critical Care Medicine and the Infectious Diseases Society of America, proposed to use PCT as an adjunctive diagnostic marker for differentiating bacterial infections from other inflammatory non-bacterial conditions (58).

Although procalcitonin is a marker of inflammation and its levels can be elevated in conditions other than sepsis, it remains the most widely used diagnostic marker of sepsis.

In sepsis, procalcitonin levels are much higher than in other inflammatory conditions. PCT levels decrease with patient's recovery, and its consistently elevated levels are reported to correlate with poor prognosis. In several studies, initial PCT levels are reported to correlate with disease severity, and can be used in disease stratification. However, some studies have not shown association of initial PCT levels and disease outcome.

1.5.1.2. Soluble CD14 subtype (sCD14-ST) – presepsin

Innate immunity cells are the first to respond to microbial invasion. Innate immune response relies on the activity of monocytes and macrophages, and is initiated by recognition of pathogens, their phagocytosis, and secretion of inflammatory cytokines. The activation of host innate immune cells is a result of their contact with membrane or structural components of pathogens, known as pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide, peptidoglycan, lipoteichoic acid, etc. Pathogen-associated molecular patterns are recognized by receptors at the

cell membrane of effector cells: monocytes and macrophages (59). After binding of PAMPs to effector cells receptors, they are consequently activated, and pro-inflammatory cytokines are secreted. These receptors are members of the Toll-like receptor (TLR) family. Two members of the Toll-like receptor family are described to participate in the process of recognition of bacterial components: TLR4 that recognizes lipopolysaccharide from Gram-negative bacteria, and TLR2 that recognizes peptidoglycan and lipoteichoic acid of Gram-positive bacteria.

CD14 (cluster of differentiation 14) is a receptor in the surface membrane of monocytes, macrophages and granulocytes, that has the ability to recognize different PAMPs in both Gram-positive and Gram-negative bacteria. Lipopolysaccharide is the most studied pathogen-associated molecular pattern.

After being released into circulation, LPS binds to a specific plasma protein known as lipopolysaccharide-binding protein (LBP). The complex lipopolysaccharide/lipopolysaccharide-binding protein (LPS/LBP) then binds to membrane-bound CD14 (mCD14) or to the free soluble form of CD14 (sCD14), thus creating a new multi-molecular complex CD14-LPS-LBP (35). CD14 intermediates presentation of LPS-LBP complex to TLR4, resulting in its activation. After the activation of TLR4, occurs the phagocytosis of bacterial components by monocytes and macrophages. The molecular complex CD14-LPS-LBP is also internalized into a phagolysosome, and submitted to an enzymatic degradation by cathepsin D. As a result of internalization and CD14 proteolysis, soluble CD14 subtype (sCD14-ST) is generated and released into circulation (60, 61).

In 2005, Yaegashi et al. (9) identified soluble CD14 subtype (sCD14-ST), later named as presepsin. They found significantly higher levels of presepsin in septic patients than in those with systemic inflammatory response syndrome (SIRS) or in healthy controls, thus introducing presepsin as a novel diagnostic marker for sepsis.

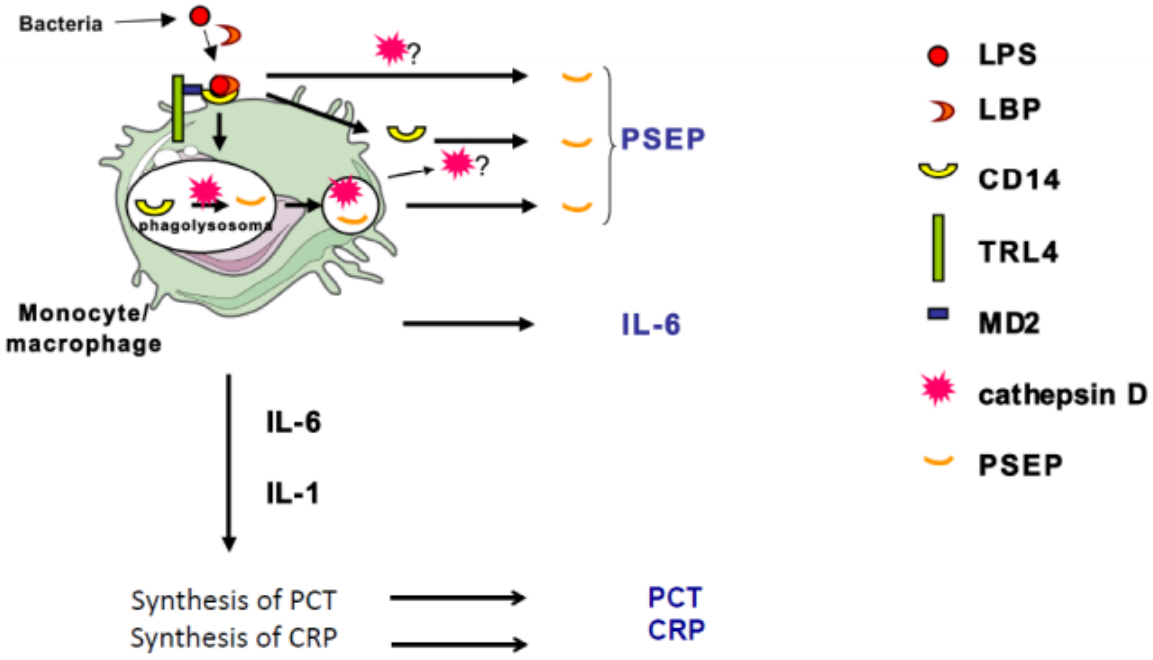


Figure 1. Mechanism of secretion of presepsin

Abbreviations: LPS-lipopolysaccharide, LBP-lipopolysaccharide-binding protein, CD14-cluster of differentiation 14, TLR4-toll-like receptor 4, MD2- myeloid differentiation factor 2, PSEP-presepsin, IL6-interleukin 6, IL1-interleukin 1, PCT-procalcitonin, CRP-C-reactive protein. Adapted from Chenevier-Gobeaux et al. (61)

Despite a large number of studies related to presepsin diagnostic role in sepsis, little is known about the mechanism of presepsin secretion. In 2015, Arai et al. (62) were the first to describe the importance of phagocytosis for presepsin secretion. They found that presepsin in humans is mainly secreted from monocytes after phagocytosis, and elastase was found as one of the essential enzymes responsible for cleaving CD14 into presepsin in monocytes. Previously, in the rabbit cecal ligation and puncture (CLP) sepsis model (63), granulocytes were found as the main source of presepsin production, and cathepsin D and asparagine protease were reported as enzymes related to CD14 cleavage.

1.5.1.2.1. Diagnostic and prognostic value of presepsin

Presepsin is an early marker of sepsis. It can be detected in the blood within hours after the onset of sepsis (64, 65). In 2008, Nakamura et al. (64) in an experimental peritonitis model of sepsis in rabbits using cecum ligation and puncture, detected presepsin in the blood of animals two hours after initiation of the procedure. Presepsin levels peaked at 3 hours, and decreased 4-8 hours after initiation of procedure. In 2016, Chenevier-Gobeaux et al. (65) measured presepsin levels after stimulation with LPS in a human cell line of monocytic cells (THP-1) and in peripheral mononuclear cells. They found presepsin concentrations elevated after 1 hour, reaching a peak after 3 hours, and decreasing at 4 hours after LPS exposure.

Starting from 2005, when presepsin was first reported to be increased in patients with sepsis, a large number of studies have been done for evaluating its diagnostic and prognostic accuracy.

By measuring presepsin concentrations in various study groups, different studies (9, 66-69) have found that presepsin is present in low concentrations in healthy individuals.

Yaegashi et al. (9) were the first to demonstrate much higher elevation of presepsin concentration in patients with sepsis than in subjects with SIRS and in healthy controls, suggesting its utility as an early diagnostic marker of sepsis. They found that in comparison to CRP and PCT, presepsin levels strongly correlate with the clinical course of sepsis. As per that study, the levels of presepsin increase earlier and at higher concentrations than CRP and PCT. Presepsin was found useful for differentiating non-infectious SIRS from sepsis and septic shock. Presepsin levels were found significantly higher in patients with sepsis, severe sepsis or septic shock than in controls (healthy individuals and SIRS group) (66-68, 70, 71).

Presepsin concentration increases with the severity of sepsis. Levels of presepsin were found to be much higher in patients with severe sepsis and septic shock than in those with sepsis (66, 68, 71), thus suggesting that presepsin concentrations could be used for severity of disease stratification.

Different studies have shown a correlation between presepsin and PCT levels in patients with sepsis. In comparison to other sepsis biomarkers, presepsin was found to be a better diagnostic marker (9, 66, 68, 71). Compared to procalcitonin, presepsin is a more specific biomarker since its production is associated with bacterial phagocytosis (9, 66).

Presepsin was reported to be useful for mortality prediction (68, 70-73). Ulla et al. (70) found a correlation between initial presepsin values and a 60-day in-hospital mortality in patients with sepsis, severe sepsis or septic shock. Analysing a 60-day mortality, they found presepsin to be superior to procalcitonin. Liu et al. (68) showed a correlation between presepsin levels in severe sepsis and septic shock, and a 28-day mortality.

Initial presepsin levels were found higher in non-survivors than in survivors (68, 70-73), thus suggesting a possible prognostic role of presepsin. In a multicenter randomized Albumin Italian Outcome Sepsis trial (ALBIOS) (72), it was reported that presepsin levels remained high over 7 days in non-survivors, and decreased over time in survivors. Higher initial presepsin levels were associated with mortality. In 2014, Masson et al. (73) compared prognostic accuracy of presepsin and procalcitonin in mortality prediction; they found presepsin to be a marker of mortality with better prognostic performance than procalcitonin. In that multicenter study, presepsin levels were found higher at baseline and over the course of disease in non-survivors, whereas procalcitonin levels did not differ between survivors and non-survivors with septic shock. Levels of procalcitonin decreased rapidly and in a similar way in both severity groups.

Combination of presepsin with prediction outcome scores increases the accuracy of mortality prediction in patients with severe sepsis and septic shock. The combination of APACHE II score with presepsin increases the accuracy in predicting septic shock (68). When compared, the prognostic accuracy of presepsin for mortality prediction was similar to that of SOFA score (73).

Presepsin levels were not found to differ between patients with Gram-positive and Gram-negative sepsis (67, 73). No significant differences were found in presepsin levels in relation to the site of infection, blood culture results (bacterial, fungal, mixed or undetermined) or the type of infection (purely Gram-positive, purely Gram-negative, mixed or undetermined) (73). Endo et al. (67) reported no significant differences in presepsin levels between blood culture positive and blood culture negative groups, thus suggesting that presepsin levels may allow a decision to provide antibiotic treatment in patients with sepsis and blood culture negative results.

In 2013, Nakamura et al. (74) measured presepsin levels in septic and sepsis-free patients with and without acute kidney injury (the AKI group and non-AKI group). Further, they divided the AKI group into those with risk, kidney injury, kidney failure, loss of kidney function, and end-stage disease. They found higher concentrations of presepsin in patients with kidney failure and advanced kidney injury, in both sepsis and non-sepsis patients. These results suggest that kidneys are the most important organ involved in presepsin elimination from the blood, and that

presepsin has a low diagnostic accuracy in patients with severely impaired kidney function and may not be a reliable marker for diagnosing sepsis. Chenevier-Gobeaux et al. (75) also found increased presepsin concentrations in patients with kidney dysfunction in a population free of any acute illness. They also found that presepsin concentrations increased with age. Differently from Chenevier-Gobeaux, Behnes et al. (71) did not find a correlation between presepsin levels and patients' age and gender.

Several studies suggest that the course of presepsin levels may be used for monitoring the effectiveness of treatment. The decrease of presepsin levels at different time points in survivors may indicate the appropriateness of treatment.

The role of presepsin in antibiotic therapy guidance still needs to be explored.

1.6. Management of sepsis

In critically ill patients, sepsis causes death as commonly as myocardial infarction, polytrauma or stroke. Mortality can be reduced by early recognition and appropriate treatment of patients who are at a greater risk for developing sepsis.

Several conditions, in which the immune system is impaired, increase the risk for developing sepsis. Patients that are at the greatest risk for developing sepsis are listed below (76):

- Patients <1 year old or >75 years old, or very frail patients,
- Patients who have impaired immune systems because of illness or drugs (chemotherapy, impaired immune function, such as those with diabetes or sickle cell disease, or patients who have had a splenectomy, those who are on long term treatment with corticosteroids, or on immunosuppressant drugs for non-malignant disorders, such as rheumatoid arthritis,
- Patients who have had surgery, or other invasive procedures, in the past 6 weeks,
- Patients with any breach of skin integrity (intravenous drug misuse, patients with indwelling lines or catheters),
- Women who are pregnant or have given birth or had a termination of pregnancy or miscarriage in the past 6 weeks.

With the goal to reduce mortality from sepsis, a Surviving Sepsis Campaign Committee was held in 2004 (77). Representatives of 11 organizations, international critical care and infectious diseases experts in the diagnosis and management of sepsis, came together in order to develop guidelines to improve the outcome in sepsis and septic shock. Surviving Sepsis Campaign guidelines were revised in 2008 and 2012.

More recently, in 2016 (52), the Surviving Sepsis Campaign Committee updated the guidelines for early management of patients with sepsis and septic shock. Recommendations for five sections were issued: hemodynamic, infection, adjunctive therapy, metabolic, and ventilation.

Further down, major recommendations of the Surviving Sepsis Campaign guidelines for management of sepsis and septic shock are summarized (52).

Decreased blood pressure, organ dysfunction and increased serum lactate are a result of sepsis-induced tissue hypoperfusion. Early appropriate fluid resuscitation is of great importance for the correction of sepsis-induced tissue hypoperfusion.

Initial fluid resuscitation should begin immediately. At least 30 mL/kg of IV crystalloids should be administered within the first 3 hours. Additional fluids, if needed, should be given after clinical examination and evaluation of physiologic variables: heart rate, blood pressure, respiratory rate, temperature, urine output, arterial oxygen saturation. The recommended initial target mean arterial pressure (MAP) is 65 mmHg in patients with septic shock who require treatment with vasopressors. Crystalloids are recommended as the fluid of choice for initial resuscitation and volume replacement. When a large amount of crystalloids is required, in addition to crystalloids, albumins can be administered.

IV antimicrobials are recommended to be initiated within one hour after recognition of sepsis or septic shock. Delayed administration of antimicrobials is related to higher mortality (78). Empiric broad spectrum therapy with one or more antimicrobials should be initiated to cover all likely pathogens, and narrowed when the causative pathogen is identified or when clinical improvement of patients with sepsis or septic shock is noted. Treatment of septic shock must include combination of at least two antimicrobial classes to cover the most likely pathogens. When clinical improvement is noticed in patients with septic shock, or when there is an evidence of infection resolution, discontinuation of combination therapy is recommended. The choice of empirical antimicrobial therapy should be based on several factors (52):

- The anatomic site of infection;

- Prevalent pathogens within the community, hospital, and even hospital ward;
- The resistance pattern of prevalent pathogens;
- The presence of specific immune defects such as neutropenia, splenectomy, poorly controlled HIV infection and acquired or congenital defects of immunoglobulin, complement or leukocyte function or production;
- Age and patient comorbidities including chronic illness (e.g. diabetes) and chronic organ dysfunction (e.g. renal or liver failure), the presence of invasive devices (e.g., central venous lines or urinary catheter) that compromise the defense to infection.

Based on all above mentioned factors, the choice of empirical antimicrobial therapy should be initiated. However, a suggestion for initial empirical antimicrobial therapy was made by the Surviving Sepsis Campaign guideline committee; a broad-spectrum carbapenem (e.g. meropenem, imipenem/cilastatin or doripenem) or extended-range penicillin/β-lactamase inhibitor combination (e.g. piperacillin/tazobactam or ticarcillin/clavulanate) were suggested as initial empirical antimicrobial therapy. Third- or higher-generation cephalosporins can also be used as a part of multidrug regimen. When there is a risk for methicillin-resistant *Staphylococcus aureus* (MRSA) infection, vancomycin, teicoplanin or another anti-MRSA agent must be administered. If there is a risk for fungal infection, an anti-fungal agent should be used (52).

Vasoactive agents should be used in refractory hypotension that does not respond to fluid resuscitation. Norepinephrine is recommended as the first-choice vasoactive agent to maintain targeted MAP. When with norepinephrine alone MAP cannot be reached, vasopressin or epinephrine can be additionally used. Dopamine can be added to norepinephrine as an alternative vasopressor in highly selected patients, those with low risk of tachyarrhythmia and absolute or relative bradycardia. When despite the use of vasopressor agents and adequate fluid replacement, patients show evidence of persistent hypoperfusion dobutamine can be administered (52).

As for sepsis-induced acute respiratory distress syndrome (ARDS), mechanical ventilation with a tidal volume of 6 mL/kg predicted body weight and higher positive end-expiratory pressure (PEEP) are recommended. In order to prevent aspiration and development of ventilator-associated pneumonia (VAP), mechanically ventilated septic patients must be maintained with the head of the bed elevated between 30 and 45 degrees (52).

Sepsis is a state that complicates severe infections and is a leading cause of death in critically ill patients (27). Among infectious diseases, sepsis is one of the most difficult to diagnose, due to its poor and highly variable clinical presentation. The early diagnosis of sepsis remains a real challenge for clinicians since a rapid diagnostic tool is still not available. Early diagnosis is crucial for a favorable outcome. Finding a biomarker that is specifically increased in septic patients at the early stage of disease would be a great advantage for clinicians.

The present study was designed to evaluate the ability of presepsin to diagnose sepsis, as well as to test its prognostic value, and to compare it with other sepsis biomarkers and scoring systems.

In this study, enrolled patients were suspected to have sepsis and diagnosed by a specialist of infectious diseases, so the possibility of misdiagnosing is smaller compared to studies with enrolment of patients in emergency departments or mixed ICUs, when sepsis is suspected by other field specialists.

We opted for testing presepsin in our patients because of its reported specific and early increase in septic patients, as well as its ability to distinguish between outcome groups. Rapid recognition of sepsis in high-risk patients and their prompt treatment may improve the outcome of disease. Compared to blood culture results, presepsin test results can be obtained rapidly, so initiation of treatment need not be delayed. Patients admitted to infectious diseases clinics are often transferred from different medical, surgical and emergency departments and treated with antibiotics prior to admission; as such, blood culture results in those patients are often negative, making the diagnosis of sepsis more difficult by using blood culture results only.

We aimed to show the importance of presepsin for identifying high-risk patients with unfavorable outcome of disease and/or poor therapeutic response. The aim was also to show the possibility of antibiotic guidance based on presepsin levels, which is crucial for patients with sepsis and septic shock.

2. HYPOTHESIS

Two hypotheses were defined before the start of the study:

1. Serum levels of presepsin in septic patients correlate with patients' outcome;
2. The concentration of presepsin in patients with sepsis is a good indicator of the appropriateness of antibiotic therapy

3. AIMS AND OBJECTIVES

3.1. Aims of the study:

- To assess the impact of presepsin concentrations on patients' outcome;

- To evaluate presepsin concentrations as a prognostic marker of antibiotic response.

3.2. Objectives of the study:

- To evaluate presepsin levels in patients with favorable and unfavorable outcome;

- To evaluate the correlation between presepsin and other biomarkers of sepsis: procalcitonin (PCT), and C-reactive protein (CRP);

- To assess the correlation between presepsin and scoring systems: Sepsis-related Organ Failure Score (SOFA) score and Acute Physiologic And Chronic Health Evaluation (APACHE II) score;

- To evaluate the impact of appropriate antibiotic treatment on presepsin levels and other biomarkers of sepsis (PCT, CRP).

4. MATERIALS AND METHODOLOGY

4.1. Study design

A prospective observational study was conducted in two university clinical centers: University Clinical Center of Kosovo, Clinic of Infectious Diseases in Pristina; and University Clinical Center of Croatia, Hospital for Infectious Diseases in Zagreb. More than half of patients were treated in the ICUs of both clinics, and the other part of enrolled patients were treated in the Department of Neuroinfections and Blood-stream Infections, at the Clinic of Infectious Diseases in Pristina.

Patients were enrolled in the study during two time periods. The first half of consecutive sepsis suspected patients were enrolled in the study between end of February 2015 and end of May 2016, and the other half between end of February 2018 and end of December 2018, accounting for a total of 100 patients.

The ICU of the Clinic of Infectious Diseases in Pristina consists of 4 beds, whereas the ICU of the Hospital of Infectious Disease in Zagreb consists of 18 beds.

After obtaining informed consent from patients or their supervisor, 100 (48 male, 52 female) consecutive sepsis suspected patients addmited to the Clinic of Infectious Diseases in Pristina, University Clinical Center of Kosovo, and Hospital for Infectious Diseases in Zagreb, University Clinical Center of Croatia, were included in the study.

Before the start of the study, the ethical approval was obtained from the Ethics Committee of both University Clinical Centers, in Pristina and in Zagreb.

All consecutive sepsis suspected patients on admission, aged ≥18 years, of both genders, previously healthy subjects and patients with history of chronic organ dysfunction (chronic kidney failure, chronic obstructive pulmonary disease, diabetes mellitus, heart failure: NYHA IV), recent surgery or recent invasive procedure, patients on immunosupressive therapy after organ transplantation with documented malignancy, were enrolled in the study.

4.1.1.Inclusion criteria

Patients' inclusion criteria were: age ≥18 years old, infection suspected or documented, and at least two of the following criteria determined by the Sepsis-3 Consensus Conference 2016 as clinical criteria for sepsis suspicion: 1) altered mentation; 2) systolic blood pressure <100 mmHg; and 3) respiratory rate ≥22 breaths/min.

4.1.2. Exclusion criteria

Patients' exclusion criteria were: age <18 years, incapability for obtaining informed consent and evidence of a different diagnosis.

After being included in the study, patients were excluded if a different diagnosis was documented: metastatic meningitis, pulmonary thromboembolism, hemorrhagic fever, leptospirosis, etc.

4.1.3. Establishment of diagnosis

Initial diagnosis was done by a constellation of clinical presentation, history for presence of risk factors, routine laboratory findings, and imaging techniques.

Clinically, sepsis was suspected in patients presenting with altered clinical state, hypotension, fever, tachypnea, tachycardia, signs and symptoms from infection site: cough, dysuria, open wounds, signs of inflammation at the central venous catheter site, or signs and symptoms from other sites of infection. Additionally, when laboratory findings showed elevated procalcitonin levels and/or C-reactive protein levels, decreased platelet count and increased creatinine levels, the initial diagnosis of sepsis was established.

In all patients with chills and body temperature higher than 37.9°C on Day 1, or at any time during the study, blood was withdrawn for culture. In all patients with obvious site of infection, site

samples were taken and cultured: urine, wound swab, cerebro-spinal fluid, etc. When intravenous catheter was a suspected site of infection, it was removed and then cultured.

Chest radiography and abdominal ultrasound were done in all enrolled patients, except in those with short stay (a few hours) and when clinical state did not allow such imaging examinations. Echocardiography was done in endocarditis suspected patients with a history of intravenous drug abuse. Other imaging techniques, such as computed tomography (CT) or magnetic resonance imaging (MRI), were conducted when there was a suspicion for intra-abdominal, intracranial, intrathoracic or soft tissue infectious focus.

When by imaging techniques, intra-abdominal or intracranial abscesses were found, patients were transferred to surgery department.

A great number of patients were transferred from emergency departments, departments of internal medicine or surgery, and a limited number of patients were transferred to our hospitals from non-university hospitals.

4.1.4. Initial treatment

All sepsis suspected patients were initially treated with fluids and empiric antimicrobial therapy, according to suspected site of infection. Initial antibiotic therapy was narrowed or changed if inappropriate according to blood or site culture results. Furthermore, antimicrobial treatment was changed in patients with negative blood and/or site culture results when a poor responsiveness to intial antimicrobial treatment was observed.

Vasoactive agents were added in all septic shock patients that did not respond to fluid replacement therapy.

Respiratory insufficiency was managed initially by oxygen therapy through nasal cannula (when SO_2≤92%), or in severe cases with acute respiratory distress syndrome by mechanical ventilation.

When diuresis was not reset after fluid therapy, and renal dysfunction was severe, central venous catheter was placed and patients were intermittently dialyzed.

Patients that needed surgical treatment of infectious focus were transferred to appropriate surgery department.

4.2. Disease stratification

Patients were stratified into sepsis group and septic shock group, according to Sepsis-3 Consensus Conference Definitions.

All consecutive sepsis suspected patients with qSOFA ≥2 on admission, were included in the study.

Patients with persistent hypotension that could not be corrected with fluid resuscitation requesting the use of vasoactive agents, were classified as septic shock patients.

4.3. Outcome based patients' grouping

Patients were followed until hospital discharge.

According to disease outcome, patients were divided into two groups: those with favorable outcome (survivors) and those with unfavorable outcome (non-survivors).

Death was considered as an unfavorable outcome.

4.4. Data collection

Demographics and clinical features, as well as laboratory parameters of enrolled patients, were collected.

The following demographic features were collected: age, gender, admission diagnosis, length of ICU stay, length of hospital stay, accompanying comorbidities, recent surgery or invasive

procedure, site of infection, antibiotic treatment prior to hospitalization, disease stratification, disease outcome, findings on chest radiography, blood and site culture findings, abdominal ultrasound findings, other imaging techniques findings, need for mechanical ventilation, need for hemodialysis, initial antibiotic treatment, length of initial antibiotic treatment, change of antibiotic during treatment, reasons for antibiotic change, length of definitive antibiotic therapy.

The following hemodynamic parameters were daily collected: mental status, heart rate, respiratory rate, ventilation status, mean arterial pressure, fever, urine output. To evaluate mental status, Glasgow Coma Scale (GCS) was calculated.

At four time points, on admission (T0), after 24 hours (T1), after 72 hours (T2) and on Day 7 (T3), the following laboratory parameters were recorded: red blood cell count, hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, serum total bilirubin, serum liver enzymes (aspartate aminotransferase AST and alanine aminotransferase ALT), partial thromboplastin time (PTT), international normalized ratio (INR), electrolytes (Na and K), blood gas analysis, C-reactive protein, procalcitonin and presepsin concentrations.

For evaluating organ dysfunction and mortality prediction, two scores were calculated: Sequential (sepsis-related) Organ Failure Assessment (SOFA) score, and Acute Physiology And Chronic Health Evaluation II score (APACHE II). Both scores were calculated at four time points: T0, T1, T2, and T3.

Routine laboratory parameters were tested immediately.

For procalcitonin and presepsin measurements, blood was collected at all four time points, frozen until the end of study, and then measured.

4.5 Sample collection for sepsis biomarker measurements

Blood samples for measuring sepsis biomarkers were collected at four time points: on admission (T0), after 24 hours (T1), after 72 hours (T2), and on Day 7 (T3).

A 5 ml blood sample was taken from cubital vein after initial skin desinfection with 70% alcohol. Blood samples were collected using sodium citrate or ethylenediamine tetraacetic acid (EDTA) as anticoagulants.

Blood was centrifuged for 15 minutes at 1000 x g within 30 minutes of collection, then serum samples were stored at -40°C for later procalcitonin and presepsin concentration testing.

4.5.1. Procalcitonin method of measurement

Procalcitonin levels were measured in each center: Hospital for Infectious Diseases in Zagreb, and in the Institute of Biochemistry in Pristina, University Clinical Centre of Kosovo.

In both centers, quantitative analysis of procalcitonin was performed using an automated electrochemiluminescence immunoanalyzer (ELECSYS* BRAHMS* PCT; Roche Diagnostics, Mannheim, Germany).

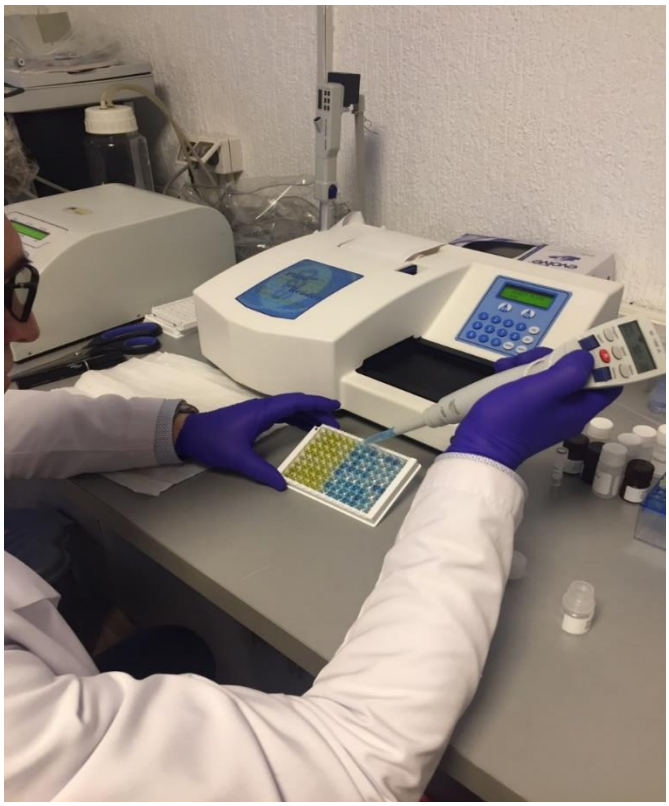
4.5.2. Presepsin method of measurement

Presepsin measurements were done in „PROLAB“ biochemical laboratory in Pristina, by professionally trained staff. ELISA kits for presepsin measurement were imported from the manufacturer Nordic Biosite based in Sweden, after obtaining permission for import from the Agency for Medicinal Products of Kosovo. Kits were used for reserach purposes only. A sandwich enzyme-linked immune-sorbent assay –Human Presepsin ELISA Kit from Nordic Biosite, was used for presepsin measurement.

Anti-presepsin antibody was pre-coated onto 96-well plates. The biotine conjugated anti-presepsin antibody was used as detection antibody. The standards, test samples and biotine conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. Horseradish-Streptavidine (HRP-Streptavidine) was added and unbound conjugates were washed away with wash buffer. Tetramethylbenzidine (TMB) substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product. When stop acidic solution was added, the blue color as a product of reaction between Horseradish Streptavidine and tetramethylbenzidine turned yellow.

The density of yellow was proportional to the presepsin amount of sample captured in plate. The O.D. absorbance at 450 nm was read in a microplate reader, and then the concentration of presepsin was calculated. Detection range of presepsin in the serum was: 0.156-10 ng/ml. After the first measurement of samples, a large number of overranged results was obtained. Therefore, serum samples were diluted in 1:10 proportion, then presepsin concentrations were measured again. Gained concentrations of presepsin were multiplied by 10 and then final results were calculated.

Picture 1. Sandwich Human Presepsin ELISA procedure; one of the multiple steps when performing the procedure



4.6. Organ dysfunction and therapy failure definitions

As recommended by Sepsis-3 Consensus Conference task force, SOFA score was used for defining organ dysfunction and organ failure. Organ dysfunction was defined as a SOFA score of 2 points, and organ failure as SOFA ≥3.

Altered mentation was defined as GCS <13 points.

Liver dysfunction and liver failure were defined as hepatic SOFA of 2 and 3 points, respectively. We defined coagulation disorders as follows: platelet count <100,000, which complies with SOFA=2, and prolonged international normalized ratio (INR >1.5) or prolonged partial thromboplastin time (PTT >80“).

Kidney injury or failure was defined according to the KDIGO criteria (79)**,** as follows:

Stage 1 acute kidney injury: serum creatinine 1.5-1.9 times baseline or ≥26.5 μmol/L increase, or urine output <0.5 ml/kg/h for 6-12 hours.

Stage 2 acute kidney injury: serum creatinine 2.0-2.9 times baseline or urine output <0.5 ml/kg/h for ≥12 hours.

Stage 3 acute kidney injury: serum creatinine 3 times baseline or ≥353.5 μmol/L increase, or initiation of renal replacement therapy, or urine output <0.3 ml/kg/h for ≥24 hours or anuria ≥12 hours.

We aimed to evaluate the association of presepsin concentrations with antibiotic therapy failure. We defined therapy failure as persistence of hypotension (MAP < 65 mmHg) and fever (temperature >37.9°C), 72 hours after initiation of antibiotic therapy.

4.7. Statistical analysis

Categorical variables were reported as frequency and percentage. Continuous variables were reported as medians, 25th and 75th percentiles, and means ± one standard deviation (±SD).

Simple comparisons were done for categorical variables using the chi-square test or Fisher's exact test as appropriate, and the Wilcoxon rank-sum test for continuous variables.

Generalized linear mixed effects model was used to test the changes in presepsin concentrations during the illness and to estimate the difference between two outcome groups (survivors and non-survivors) as well as between two severity groups (sepsis and septic shock), after adjustments for baseline presepsin values. Receiver Operating Characteristic (ROC) curves and areas under the ROC curves (AUCs) were calculated to test the importance of initial presepsin concentrations on sepsis outcome and sepsis severity. Based on optimal cut-off values of presepsin for discriminating between outcome groups and severity groups, according to ROC curve analysis, the sensitivity and specificity of the found threshold values were calculated.

Longitudinal analysis using generalized linear mixed effects modelling was performed to test the association of initial SOFA and APACHE II scores with poor outcome, after adjustment for initial values and day of illness. Generalized mixed effects model was used to test the changes in APACHE II and SOFA scores during the illness and to estimate the difference between two severity groups after an adjustment for baseline values. Adjustment was done because baseline score values have a strong impact on subsequent values.

To test the impact of initial CRP values on patients' outcome, multivariate analysis was used, after adjustment for timing and initial CRP values. Because of a number of procalcitonin missing values, generalized linear mixed effects model was not appropriate due to the lack of convergence. Finally, multivariate logistic regression analysis was performed to test the association of increased biomarkers with outcome.

To evaluate the association of therapy failure with initial presepsin values, Glimix procedure was used. Multivariate analysis was performed to assess the association of presepsin values and SOFA and APACHE II scores, after an adjustment for day of hospitalization. For all statistical tests, significance was set at an alpha level of 0.05. All analyses were performed using SAS software version 9.3 (SAS Institute, Cary, North Carolina, USA).

5. RESULTS

5.1. Patients' demographics and clinical features

From a total of 116 sepsis suspected patients on admission, 100 patients from both centres, Clinic of Infectious Diseases, University Clinical Centre of Kosovo, and Hospital for Infectious Diseases, University Clinical Centre of Zagreb, were included in the study. After initial suspicion of sepsis, and later documentation of another disease, 16 patients were excluded from the study (3 patients with documented Crimean Congo haemorrhagic fever, 2 patients with leptospirosis, 1 patient with carcinomatous meningitis, 5 patients with salmonella enteritis without bacteremia, 3 patients with pyelonephiritis, and 2 patients with pancreatitis).

All included patients were sepsis suspected patients on admission or later during the hospital stay. When included in the study, patients had a qSOFA ≥2 on admission or later during the hospital stay.

According to disease outcome, patients were divided into two groups, those with favorable and unfavorable outcome. Thirty two patients who died over the course of the disease were grouped into the unfavorable outcome group (non-survivors). Patients who survived (n=68) were grouped into the favorable outcome group (survivors).

Demographic characteristics of two sepsis outcome groups (survivors and non-survivors) are shown in Table 4 and 4a.

Mortality increased with age. Mean age of patients who did not survive was 66.8 years (SD±14.0), whereas the mean age of patients who survived was 59.7 years (SD±17.2) (p=0.066).

Mortality was higher among males; 19/48 (59.4%) male patients died over the course of the disease, compared to 13/52 (40.6%) female patients.

Respiratory tract was the most common site of infection (40%), followed by genitourinary tract infections (27%), intra-abdominal infections (8%), and skin and soft tissue infections (8%). Mortality was higher in patients with pulmonary site of infection (chi-square test, p-value <0.004);

53.1% of patients who died had pneumonia as a site of infection. In 5% of patients the site of infection was unknown (Table 4).

The most common risk factor was diabetes; 45% of patients included in the study had diabetes. Diabetes was the most common risk factor among non-surviving patients (50%).

Other reported risk factors were: chronic obstructive pulmonary disease (9%), regular hemodialysis (7%), recent surgery (7%), heart failure, NYHA IV (6%), recent invasive procedures (6%), documented carcinoma (2%), and immunosuppressive therapy (2%) (Table 4a).

Table 4. Basic demographic data in two outcome groups

	Survivors (N=68)	Non-survivors (N=32)	Overall (N=100)	p-value
AGE (years)				0.066
N	68	32	100	
Median	64.5	69.0	66.0	
25th percentile	49.0	61.5	52.5	
75th percentile	72.0	78.5	74	
GENDER				0.118
Male	29 (42.6%)	19 (59.4%)	48 (48.0%)	
Female	39 (57.4%)	13 (40.6%)	52 (52.0%)	
SITE OF INFECTION				0.004
Respiratory tract	23 (33.8%)	17 (53.1%)	40 (40.0%)	
Genitourinary tract	26 (38.2%)	1 (3.1%)	27 (27.0%)	
Intravascular	3 (4.4%)	3 (9.4%)	6 (6.0%)	
Intra-abdominal	5 (7.4%)	3 (9.4%)	8 (8.0%)	
Skin and soft tissues	4 (5.9%)	4 (12.5%)	8 (8.0%)	
Post invasive procedure	1 (1.5%)	1 (3.1%)	2 (2.0%)	
CNS	3 (4.4%)	1 (3.1%)	4 (4.0%)	
Unknown	3 (4.4%)	2 (6.3%)	5 (5.0%)	

Abbreviations: SD: standard deviation; CNS: central nervous system

Patients' clinical characteristics on admission are listed in Table 5. More than half of patients had no body temperature higher than 37.9°C (54.0%) on admission and had no hypotension, defined as MAP <65 mmHg (58.0%). Shock was present on admission in 19.0% of enrolled patients. Tachycardia, defined as heart rate ≥90 beats per minute, and tachypnea defined as respiratory rate ≥22 breaths per minute, were the most common clinical findings. Altered mentation defined as GCS ≤13, was present on admission in almost half of included patients.

Overall GCS (mean ± SD) was 11.2±4.2, significantly lower in non-survivors compared to survivors, 7.3±4.6 vs. 13.0±2.3, respectively (p<0.001). Kidney injury, as defined by KDIGO (79), was present on admission in 45.0% of our septic patients (Table 6). We defined kidney failure as kidney injury KDIGO stage 2 and stage 3, and we found kidney failure to be present on admission in 28.0% of our septic patients. Liver injury and liver failure on admission were present in 17.0% and 4.0% of patients, respectively (Table 6).

Table 4a. Basic demographic data in two outcome groups (continued)

	Survivors (N=68)	Non-survivors (N=32)	Overall (N=100)	p-value
RISK FACTORS				0.395
Documented risk factor	43 (63.2%)	22 (68.7%)	65 (65.0%)	
No evident risk factor	25 (36.8%)	10 (31.3%)	35 (35.0%)	
DIABETES MELLITUS				0.524
Documented DM	29 (42.6%)	15 (46.9%)	44 (44.0%)	
No history of DM	39 (57.4%)	17 (53.1%)	56 (56.0%)	
HEMODIALISYS				1.000
Regular hemodialysis	5 (7.4%)	2 (6.3%)	7 (7.0%)	
No history of chronic renal failure	63 (92.6%)	30 (93.8%)	93 (93.0%)	
COPD				0.028
Chronic obstructive pulmonary disease	3 (4.4%)	6 (18.8%)	9 (9.0%)	
No history of COPD	65 (95.6%)	26 (81.3%)	91 (91.0%)	
NYHA IV				0.330
Heart failure NYHA IV	3 (4.4%)	3 (9.4%)	6 (6.0%)	
No history of heart disease	65 (95.6%)	29 (90.6%)	94 (94.0%)	
CARCINOMA				0.581
Documented carcinoma	1 (1.5%)	1 (3.1%)	2 (2.0%)	
No history of malignancies	67 (98.5%)	31 (96.9%)	98 (98.0%)	
IMMUNOSUPPRESSION				0.581
Patients on immunosuppressive therapy	1 (1.5%)	1 (3.1%)	2 (2.0%)	
Patients with no history of immunosuppression	67 (98.5%)	31 (96.9%)	98 (98.0%)	
SURGERY				0.523
Recent surgery	4 (5.9%)	3 (9.4%)	7 (7.0%)	
No history of recent surgery	64 (94.1%)	29 (90.6%)	93 (93.0%)	
INVASIVE PROCEDURE				0.942
Recent invasive procedure	4 (5.9%)	2 (6.3%)	6 (6.0%)	
No history of recent invasive procedure	64 (94.1%)	30 (93.8%)	94 (94.0%)	

Abbreviations: DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; NYHA IV: New York Heart Association class IV

Thrombocytopenia (PLT <100,000) on admission was present in 23.0% of patients, prolonged INR or PTT in 13.0%, and the combination of platelet count <100,000 and prolonged INR or PTT was found in 6.0% of patients. Additionally, 20.0% of all ICU treated patients with sepsis were mechanically ventilated.

Table 5. Clinical presentation on admission

Vital parameters		Overall
GCS ≤13	n	46
	%	46.0
Body temperature ≥38°C	n	46
	%	46.0
Heart rate ≥90 beats/min	n	83
	%	83.0
Respiratory rate ≥22 breaths/min	n	82
	%	82.0
MAP <65 mmHg	n	23
	%	23.0
MAP <65 mmHg + need for vasoactive agents	n	19
	%	19.0

Abbreviations: GCS: Glasgow coma scale; MAP: mean arterial pressure

Table 6. Organ dysfunction parameters on admission

Organ dysfunction parameters		Overall
Kidney injury		
Stage 1 KDIGO criteria	n	*17*
	%	*17.0*
Stage 2 KDIGO criteria	n	*16*
	%	*16.0*
Stage 3 KDIGO criteria	n	*12*
	%	*12.0*
Liver injury		
Hepatic SOFA=2	n	*17*
	%	*17.0*
Hepatic SOFA≥3	n	*4*
	%	*4.0*
Coagulation disorders		
Platelet count <100.000	n	*23*
	%	*23.0*
PTT>80" or INR>1.5	n	*13*
	%	*13.0*
Platelet <100.000 + prolonged INR or PTT	n	*6*
	%	*6.0*
Mechanical ventilation	n	*20*
	%	*20.0*

Abbreviations: KDIGO-Kidney disease improving global outcomes; SOFA- Sequential (Sepsis-related) organ dysfunction assessment; PTT-partial thromboplastin time; INR-International normalized ratio

5.2. Clinical diagnosis of sepsis

In most patients with blood and site culture negative results, clinical signs of infection were evident or the infectious focus was found by imaging techniques.

Pneumonia was the most frequent site of infection with no microbiological documentation. Patients had clinical symptoms and imaging signs of pneumonia in 28.0% without microbiological documentation. Pyelonephritis was diagnosed according to abdominal ultrasound, abdominal CT and urine findings. Pyelonephritis without microbiological documentation was found in 17.0% of overall patients. Abdominal and pulmonary abscesses were diagnosed according to CT findings. Soft tissue infections were diagnosed according to inflammatory signs at the site of infection and culture of wound swabs. Peritonitis was diagnosed based on ultrasound and CT findings. There were two patients that developed sepsis after an invasive procedure and had no documented infection; one patient had previously undergone an abortion, and the other a cystoscopy.

5.3. Sepsis diagnosis by blood and/or site cultures

Infection was microbiologically documented in 35% of patients, according to blood and site culture results. Forty-eight percent of patients were treated with antibiotics prior to hospitalization. Polymicrobial etiology of sepsis was recorded in 7/35 (20%) patients with microbiologically documented infection.

Blood and site cultures results are shown in Table 7 and Table 7a. Blood cultures were taken from all patients with body temperature higher than 37.9°C on admission or later during the stay when they presented fever.

Gram-positive bacteria were more often isolated from blood compared to Gram-negative bacteria. Gram-positive and Gram-negative bacteria accounted for 53.8% vs. 42.4% of isolates, respectively. We identified polymicrobial blood culture-based etiology in 3.8% of our septic patients.

Table 7. Microbiological documentation of infection

	(n) ***Number of positive isolates***	**(%)** ***Percentage of positive isolates***
Blood and site culture results		
Blood culture results	20	57.2
Site culture results	5	14.3
Blood + site culture identical results	4	11.4
Blood + site culture different results	2	5.7
Different results from different site cultures	4	11.4
Polymicrobial etiology	7	20.0
Blood culture isolates		
Gram positive bacteria:	14	53.8
Staphylococcus aureus	6	
Coagulase negative Staphylococcus	6	
Streptococcus β-haemolyticus group A	2	
Gram negative bacteria:	11	42.4
Escherichia coli	9	
Pseudomonas spp.	1	
Proteus mirabilis	1	
Polymicrobial	1	3.8

Table 7a. Site culture results

	(n) *Number of positive isolates*	(%) *Percentage from site culture isolates*
Urine culture isolates:		
Escherichia coli	5	26.3
Enterococcus spp.	2	10.5
Klebsiella pneumoniae	1	5.3
Staphylococcus aureus	1	5.3
Candida spp.	1	5.3
Culture or PCR of CSF:		
Streptococcus pneumoniae	2	10.5
Staphylococcus aureus	1	5.3
Ezakiella spp.	1	5.3
Culture of nasopharyngeal swab or aspirate:		
group A Streptococcus	1	5.3
Staphylococcus aureus	1	5.3
Acynetobacter spp.	1	5.3
Culture of punctate of abscess:		
polymicrobial: Proteus spp., Streptococcus intermedius	1	5.3
Culture of wound swab:		
polymicrobial: Enterococcus faecalis, Acynetobacter spp.	1	5.3

Abbreviations: PCR-polimerase chain reaction; CSF-cerebrospinal fluid

5.4. Presepsin values during study

We assessed presepsin concentrations four times over the course of the disease (Table 8, Figure 2). Presepsin levels were compared between survivors and non-survivors.

At all time periods, presepsin concentration was significantly higher in non-survivors compared to survivors, thus showing its prognostic value. High presepsin concentrations on admission that did not decrease rapidly were associated with poor outcome (Figure 2).

In non-survivors, presepsin concentration remained high until the end of disease or until last measurement. Presepsin concentration decreased slowly in non-survivors and remained at least 4-fold above the upper reference range on Day 7, whereas in survivors presepsin concentration decreased more rapidly, and on Day 7 returned within the reference range.

Table 8. Presepsin concentrations (ng/ml) during study in two outcome groups

	Survivors	***Non-survivors***	***Overall***		***Survivors***	***Non-survivors***	***Overall***
	Presepsin on admission (ng/mL)				***Presepsin after 72 hours (ng/mL)***		
N	68	32	100	**N**	66	18	84
Median	103.6	117.7	106.9	**Median**	32.1	83.9	47.3
25th percentile	28.7	93.4	43.6	**25th percentile**	8.2	44.4	9.7
75th percentile	130.5	153.6	140.8	**75th percentile**	117.7	133.3	118.4
	Presepsin after 24 hours (ng/mL)				***Presepsin on Day 7 (ng/mL)***		
N	67	25	92	**N**	63	12	75
Median	88.4	118.8	105.4	**Median**	9.7	49.1	13.2
25th percentile	19.7	91.6	33.9	**25th percentile**	2.5	17.7	3.9
75th percentile	126.8	137.9	132	**75th percentile**	33.4	56.4	43.4

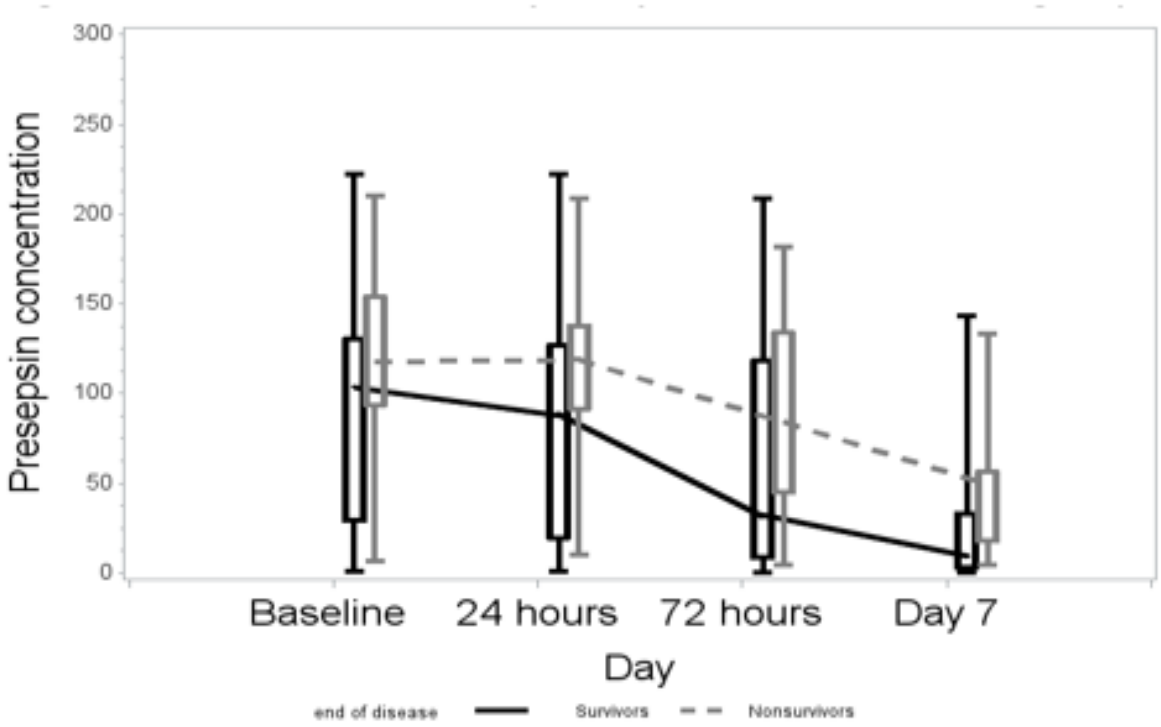


Figure 2. Concentrations of presepsin (ng/ml) in two outcome groups

Black line: survivors; black dotted line: non-survivors. The vertical left side of the figure shows presepsin concentrations expressed in ng/ml. The lower horizontal line shows presepsin concentrations at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

Generalized linear mixed effects model was used to test the changes in presepsin levels during the illness and to estimate the difference between two outcome groups. Adjustments were made with generalized linear mixed effects model for baseline presepsin values. Figure 2 shows that presepsin concentrations significantly decreased during the trial (<0.0001) and that outcome groups differed significantly (p=0.0152).

Higher presepsin values and slower decrease of presepsin concentrations were associated with increased mortality. Adjustments were done because baseline presepsin values have a strong impact on subsequent values (p<0.0001).

In the following Figure, we can notice that more patients in non-survivors group (40.6%) had values of presepsin ≥90 ng/ml. Vertical line shows threshold value of 90 ng/ml (Figure 3).

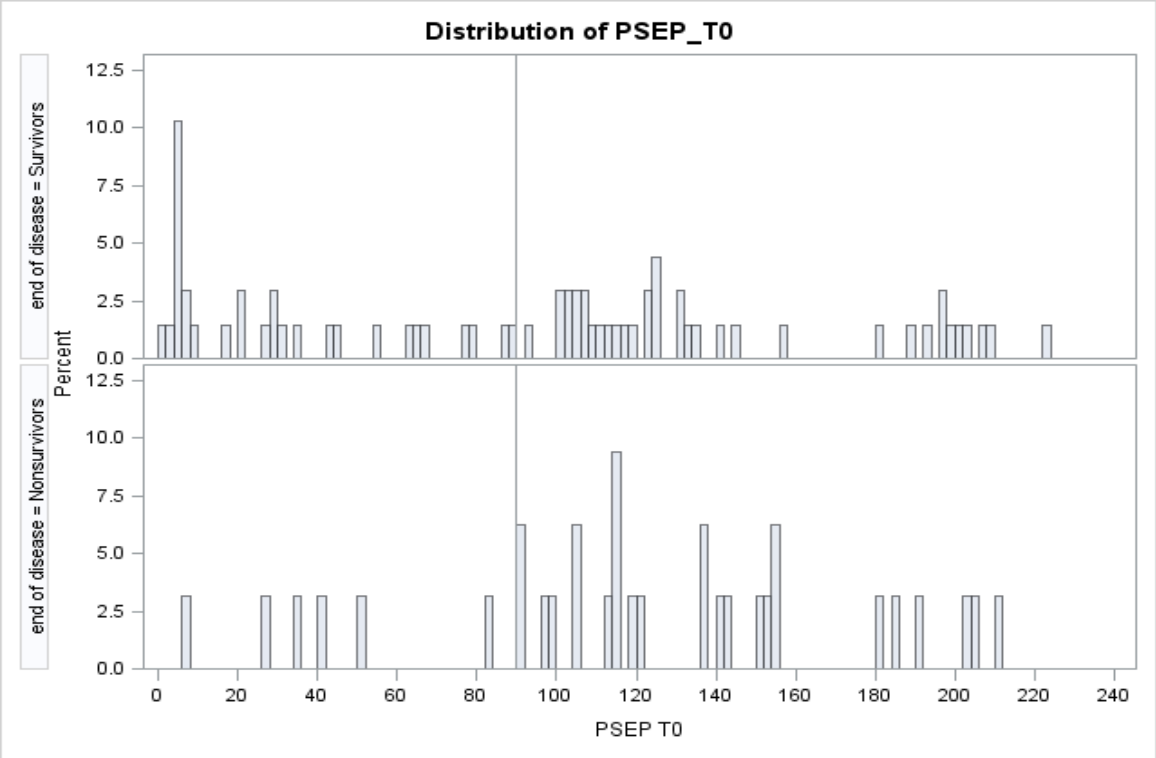


Figure 3. Distribution of presepsin concentrations on admission in two outcome groups

Abbreviations: PSEP-presepsin; T0-on admission; vertical line in the middle of the figure-threshold value of 90 ng/ml. The upper left half of the figure shows percentage of survivors at the corresponding presepsin value. The lower left half of the figure shows percentage of non-survivors at the corresponding presepsin value. The horizontal line shows presepsin values on admission, expressed in ng/ml.

To test the importance of initial presepsin values on diseases outcome, the Receiver Operating Characteristic (ROC) curve was constructed.

The ROC curve showed limited predictive value of presepsin concentrations on admission.

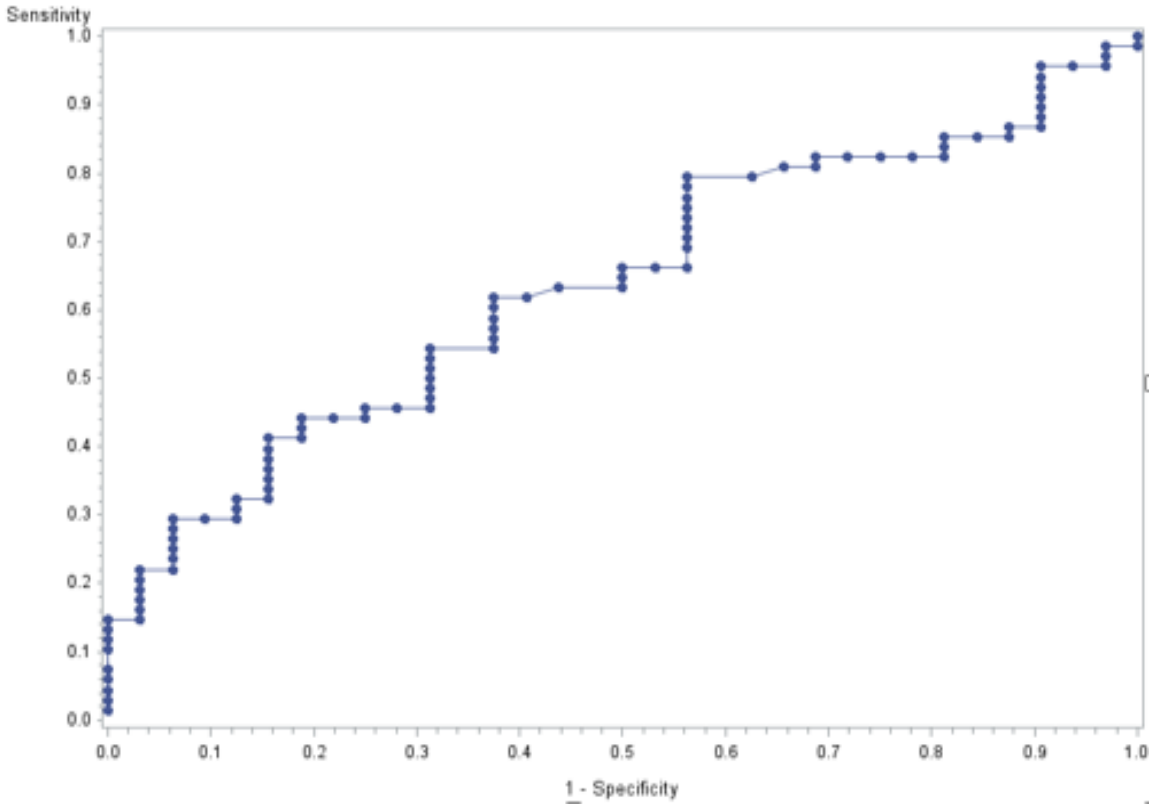


Figure 4. The Receiver Operating Characteristic (ROC) curve for presepsin concentration of ≥90 ng/ml on admission and disease outcome

Table 9. Presepsin cut-off concentration of 90 ng/ml by disease outcome

Presepsin concentration of 90 ng/ml	**Disease outcome**		
	Survivors	**Non-survivors**	**Total**
Sensitivity			
Number of patients	30	6	36
Percentage of patients	30.00	6.00	36.00
Patients with presepsin values<90 ng/ml (%)	83.33	16.67	
Sensitivity of presepsin values≥90 ng/ml	**44.12**	18.75	
Specificity			
Number of patients	38	26	64
Percentage of patients	38.00	26.00	64.00
Patients with presepsin values >90 ng/ml (%)	59.38	40.63	
Specificity of presepsin values≥90 ng/ml	55.88	**81.25**	
Total	68	32	100
	68.00	32.00	100.00

Table 9 shows high specificity (0.812) of values above 90 ng/ml, but low sensitivity (0.441), indicating that a substantial number of non-survivors had presepsin values lower than 90 ng/ml Six out of thirty-six patients (16.7%) who died had presepsin values lower than 90 ng/ml, whereas in patients with presepsin values ≥90 ng/ml (26/64), mortality was 40.6% (Fisher's exact test, p=0.0150).

5.5. Association of presepsin concentrations with severity of sepsis

Furthermore, we compared presepsin concentrations at four time points to assess if increased presepsin concentrations were associated with the severity of sepsis (Table 10 and Figure 5).

Table 10. Presepsin concentrations (ng/ml) during study in two severity groups

	Sepsis	***Septic shock***	***Overall***		***Sepsis***	***Septic shock***	***Overall***
	Presepsin on admission (ng/mL)				***Presepsin after 72 hours (ng/mL)***		
N	66	34	100	**N**	60	24	84
Median	92.3	127.2	106.9	**Median**	29.4	85.3	47.3
25th percentile	28.2	106	43.6	**25th percentile**	7.3	39.7	9.7
75th percentile	125.4	154.3	140.8	**75th percentile**	111.1	128.3	118.4
	Presepsin after 24 hours (ng/mL)				***Presepsin on Day 7 (ng/mL)***		
N	62	30	92	**N**	57	18	75
Median	66.3	122.1	105.4	**Median**	9.7	15.7	13.2
25th percentile	19.7	111.1	33.9	**25th percentile**	2.5	8.4	3.9
75th percentile	126.2	152.9	132	**75th percentile**	43.4	33.4	43.4

Generalized linear mixed effects model was used to test the changes in presepsin levels during the illness and to estimate the difference between two severity groups (sepsis and septic shock). Adjustments were done with generalized linear mixed effects model for baseline presepsin values. Figure 5 shows that presepsin concentrations significantly decreased during the trial p<0.0001) and that severity groups differed, although marginally (p=0.0459). Figure 5 also shows that differences were greater after 24 and 72 hours. Adjustment was done because baseline presepsin

values have a strong impact on subsequent values. Higher initial presepsin values and slower decrease of presepsin concentrations were marginally associated with the severity of clinical presentation.

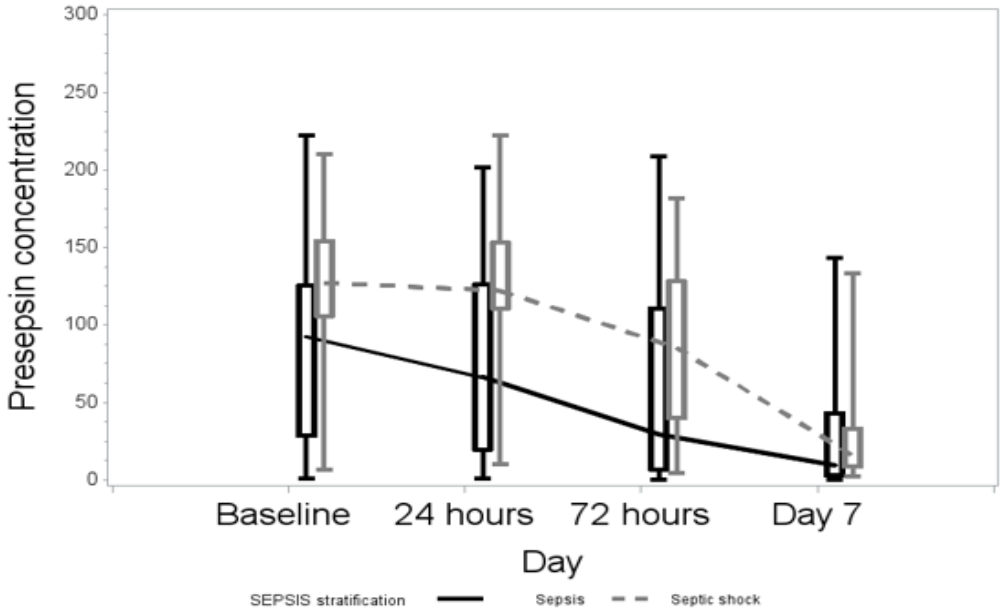


Figure 5. Presepsin concentrations (ng/ml) during study in two severity groups

Black line: sepsis patients; black dotted line: septic shock patients. The vertical left side of the figure shows presepsin concentrations expressed in ng/ml. The lower horizontal line shows presepsin concentrations at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

In Figure 6, we can notice that more patients in the shock group had values above 110 ng/ml. Vertical line shows threshold value of 110 ng/ml (Figure 6).

To test the importance of initial presepsin values on severity of sepsis the ROC curve was constructed.

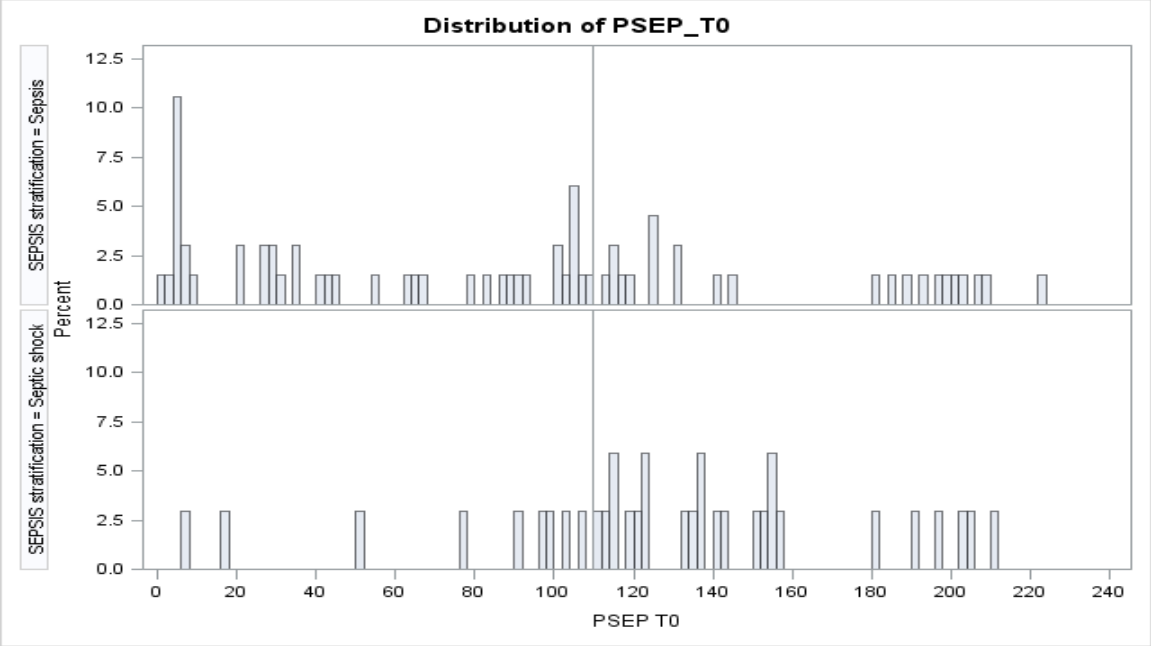


Figure 6. Distribution of presepsin concentrations on admission in two severity groups

Abbreviations: PSEP-presepsin; T0-on admission; vertical line in the middle of the figure-threshold value of 110 ng/ml. The upper left half of the figure shows percentage of patients with sepsis at the corresponding presepsin value. The lower left half of the figure shows percentage of patients with septic shock at the corresponding presepsin value. The horizontal line shows presepsin values on admission, expressed in ng/ml.

We compared the severity of illness between patients who had presepsin values ≥110 ng/ml, and those who had values of presepsin <110 ng/ml.

Table 11 shows high sensitivity (0.727) of presepsin values above 110 ng/ml, but lower specificity (0.617), indicating that patients with values ≥110 ng/ml are at a high risk of developing septic shock.

We constructed the ROC curve showing the prognostic value of presepsin, which was significantly associated with septic shock, particularly values above 110 ng/ml (Figure 7).

The horizontal line shows sensitivity rate of 0.727, and the vertical line specificity of 0.617. We can notice a sharp increase in specificity with values above 110 ng/ml. The calculated area under the ROC curve (AUC) was 0.703, showing that presepsin values explain about 70% of results.

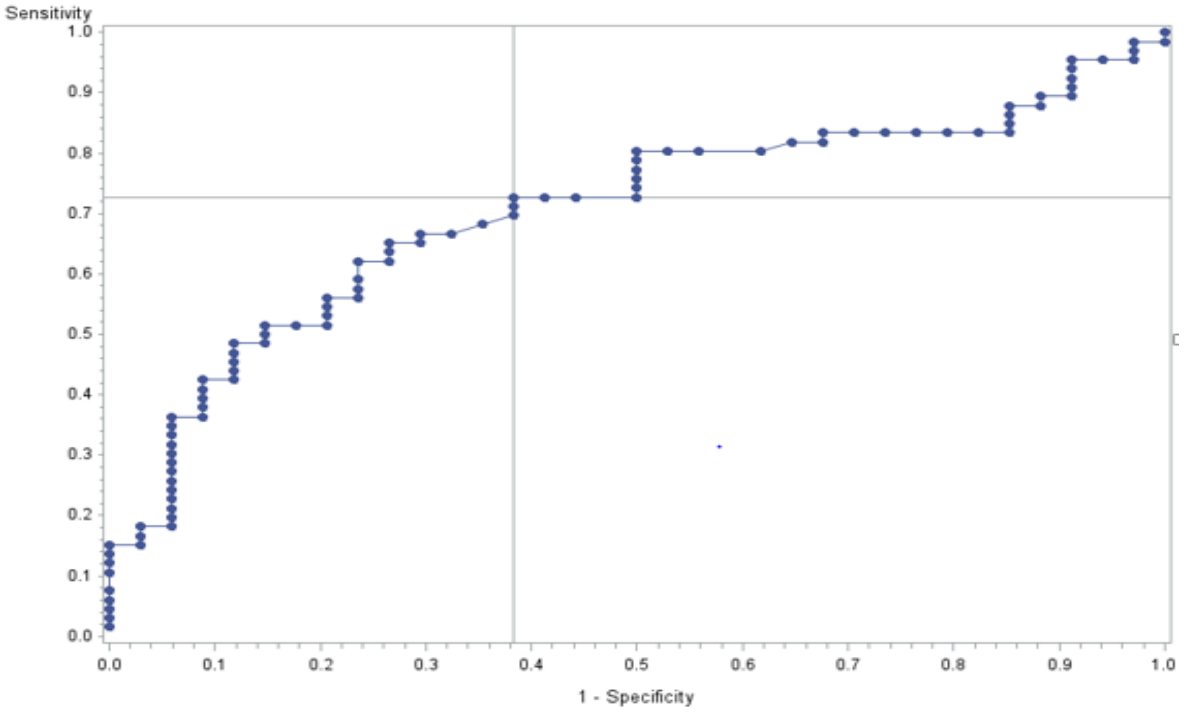


Figure 7. Receiver Operating Characteristic (ROC) curve for presepsin values and severity of sepsis

Table 11. Presepsin cut-off concentration of 110 ng/ml by disease stratification

Presepsin concentration of 110 ng/ml	**Sepsis starification**		
	Sepsis	**Septic shock**	**Total**
Sensitivity			
Number of patients	48	13	61
Percentage of patients	48.00	13.00	61.00
Patients with presepsin values<110 ng/ml (%)	78.69	21.31	
Sensitivity of presepsin values≥110 ng/ml	**72.73**	38.24	
Specificity			
Number of patients	18	21	39
Percentage of patients	18.00	21.00	39.00
Patients with presepsin values>110 ng/ml (%)	46.15	53.85	
Specificity of presepsin values≥110 ng/ml	27.27	**61.76**	
Total	66	34	100
	66.00	34.00	100.00

Thirteen out of 61 patients (21.3%) with presepsin values lower than 110 ng/ml developed septic shock, whereas 21/39 patients (53.9%) with presepsin values ≥110 ng/ml developed septic shock (Fisher's exact test, p=0.0003).

5.6. Associations of SOFA score and APACHE II score with poor outcome

Associations of disease outcome with SOFA score, and APACHE II score are shown In Table 12. Both severity scores frequently used in ICU patients were, as expected, significantly higher in non-survivors on admission as well as the worst score during hospitalization. In patients who on admission met the criteria for sepsis but during the hospital stay developed septic shock, worst values of SOFA and APACHE II scores were recorded later, during the illness, and they differed from initial values of SOFA and APACHE II scores, so we compared both, the initial and the worst values of SOFA and APACHE II scores, between outcome groups as well as between severity groups. From all four measurements of SOFA and APACHE II score in both study groups (outcome groups and sepsis severity groups), we evaluated the association of outcome and sepsis severity with the initial value and the worst value of each score (SOFA and APACHE II score) recorded during the study, regardless of day of measurement.

Table 12. Associations of scoring systems with sepsis outcome

	Survivors	***Non-survivors***	***Overall***		***Survivors***	***Non-survivors***	***Overall***
	Initial SOFA score				***Initial APACHE II score***		
N	68	32	100	**N**	68	32	100
Median	5.0	10.5	7.0	**Median**	20.0	32.5	22.5
25th percentile	3.5	7.5	4.0	**25th percentile**	14.0	22.0	17.0
75th percentile	7.5	12.5	9.0	**75th percentile**	25.0	37.0	30.0
	The worst SOFA score				***The worst APACHE II score***		
N	68	32	100	**N**	68	32	100
Median	20.0	32.5	22.5	**Median**	22.0	35.5	25.0
25th percentile	14.0	22.0	17.0	**25th percentile**	18.0	29.5	20.0
75th percentile	25.0	37.0	30.0	**75th percentile**	26.0	40.5	33.5

Abbreviations: SOFA-Sequential (sepsis-related) Organ Failure Assessment, APACHE-Acute Physiology And Chronic Health Evaluation

Interestingly, biomarkers associated with inflammatory response were not associated with poor outcome (Table 13).

Table 13. Associations of initial values of C-reactive protein and procalcitonin with disease outcome

	Survivors	***Non-survivors***	***Overall***		***Survivors***	***Non-survivors***	***Overall***
	CRP on admission				***PCT on admission***		
N	68	32	100	**N**	68	32	100
Median	197.0	199.0	197.0	**Median**	8.5	9.0	8.5
25th percentile	121.3	141.5	128.0	**25th percentile**	1.7	4.2	2.9
75th percentile	250.0	278.8	259.5	**75th percentile**	36.5	21.0	29.0

Abbreviations: CRP-C-reactive protein, PCT-procalcitonin

Dynamics of SOFA and APACHE II score throughout the study are shown in Tables 14 and 15, and Figures 8 and 9. Both parameters were significantly associated with patients' outcome.

Table 14. Dynamics of SOFA score throughout the study in two outcome groups

	Survivors	***Non-survivors***	***Overall***		***Survivors***	***Non-survivors***	***Overall***
	SOFA score on admission				***SOFA score after 72 hours***		
N	68	32	100	**N**	66	18	84
Median	5.0	10.5	7.0	**Median**	3.0	12.0	4.0
25th percentile	3.5	7.5	4.0	**25th percentile**	2.0	8.0	2.0
75th percentile	7.5	12.5	9.0	**75th percentile**	6.0	14.0	8.0
	SOFA score after 24 hours				***SOFA score on Day 7***		
N	67	25	92	**N**	63	12	75
Median	4.0	12.0	6.0	**Median**	2.0	11.0	3.0
25th percentile	3.0	8.0	4.0	**25th percentile**	1.0	7.0	1.0
75th percentile	7.0	13.0	9.0	**75th percentile**	4.0	16.0	6.0

Abbreviations: SOFA-Sequential (sepsis-related) Organ Failure Assessment

SOFA score differed significantly between survivors and non-survivors. It decreased in survivors, while remaining high in non-survivors (Figure 8).

Longitudinal analysis using generalized linear mixed effects modelling confirmed association of poor outcome with elevated SOFA values throughout the study ($p<0.0001$) after adjustment for initial values and day of illness.

Table 15. Dynamics of APACHE II score throughout the study in two outcome groups

	Survivors	***Non-survivors***	***Overall***		***Survivors***	***Non-survivors***	***Overall***
	APACHE II score on admission				***APACHE II score after 72 hours***		
N	68	32	100	**N**	66	18	84
Median	20.0	32.5	22.5	**Median**	14.0	26.0	17.0
25th percentile	14.0	22.0	17.0	**25th percentile**	11.0	24.0	12.0
75th percentile	25.0	37.0	30.0	**75th percentile**	22.0	32.0	25.0
	APACHE II score after 24 hours				***APACHE II score on Day 7***		
N	67	25	92	**N**	63	12	75
Median	18.0	31.0	20.0	**Median**	11.0	30.0	13.0
25th percentile	15.0	22.0	16.5	**25th percentile**	6.0	25.5	7.0
75th percentile	22.0	39.0	25.5	**75th percentile**	18.0	34.5	20.0

Abbreviations: APACHE-Acute Physiology And Chronic Health Evaluation

Surviving patients had a lower APACHE II score than non-survivors (Figure 9). In survivors, APACHE II score decreased over time, whereas in non-survivors APACHE II score remained high or increased over the course of the disease. Longitudinal analysis using generalized linear mixed effects modelling confirmed association of poor outcome with elevated APACHE II values ($p<0.0001$) throughout the study after adjustment for initial values and day of illness.

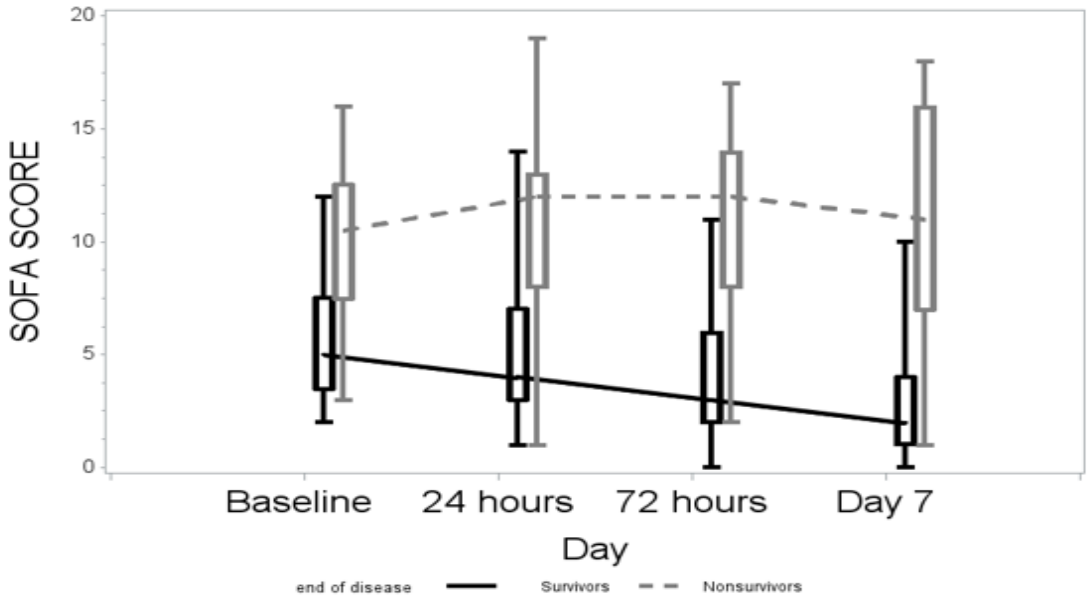


Figure 8. SOFA score values during study in two outcome groups

Black line: survivors; black dotted line: non-survivors. The vertical left side of the figure shows values of SOFA score. The lower horizontal line shows values of SOFA score at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

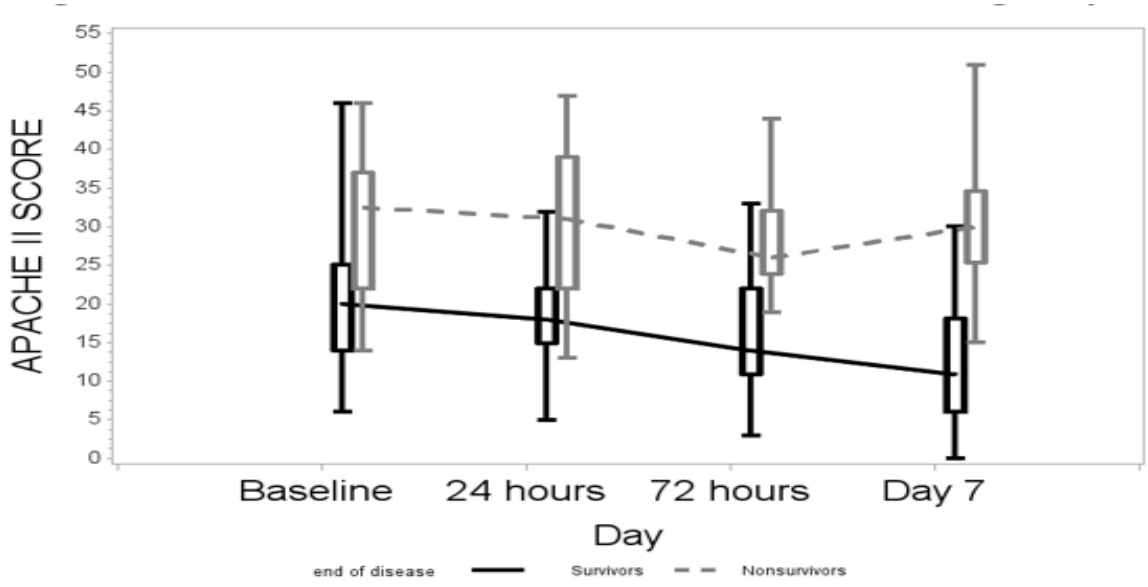


Figure 9. APACHE II score values during study in two outcome groups

Black line: survivors; black dotted line: non-survivors. The vertical left side of the figure shows values of APACHE II score. The lower horizontal line shows values of APACHE II score at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

5.7. Associations of SOFA score and APACHE II score with severity of disease

Associations of disease severity with SOFA score and APACHE II score are shown in Table 16.

Table 16. Initial and worst values of SOFA and APACHE II score in two severity groups

	Sepsis	***Septic shock***	***Overall***		***Sepsis***	***Septic shock***	***Overall***
	Initial SOFA score				***Initial APACHE II score***		
N	66	34	100	**N**	66	34	100
Median	5.0	9.5	7.0	**Median**	20.0	29.5	22.5
25th percentile	3.0	8.0	4.0	**25th percentile**	14.0	21.0	17.0
75th percentile	7.0	12.0	9.0	**75th percentile**	26.0	37.0	30.0
	The worst SOFA score				***The worst APACHE II score***		
N	66	34	100	**N**	66	34	100
Median	6.0	13.0	8.0	**Median**	22.0	34.0	25.0
25th percentile	4.0	10.0	5.0	**25th percentile**	18.0	26.0	20.0
75th percentile	8.0	15.0	11.0	**75th percentile**	28.0	40.0	33.5

Abbreviations: SOFA-Sequential (sepsis-related) Organ Failure Assessment, APACHE-Acute Physiology And Chronic Health Evaluation

As expected, both SOFA and APACHE II scores on admission and their worst values were significantly associated with the severity of disease, whereas such an association was not found for C-reactive protein and procalcitonin (Table 17).

Table 17. Associations of initial values of CRP and PCT with severity of sepsis

	Sepsis	***Septic shock***	***Overall***		***Sepsis***	***Septic shock***	***Overall***
	CRP on admission				***PCT on admission***		
N	66	34	100	**N**	66	34	100
Median	196.9	198.5	197.0	**Median**	6.0	14.2	8.5
25th percentile	125.6	138.5	128.0	**25th percentile**	2.3	4.3	2.9
75th percentile	252.2	264.5	259.5	**75th percentile**	21.9	48.8	29.0

Abbreviations: CRP: C-reactive protein, PCT-procalcitonin

Table 16 and figures 10 and 11 show the association of SOFA score and APACHE II score with the severity of sepsis.

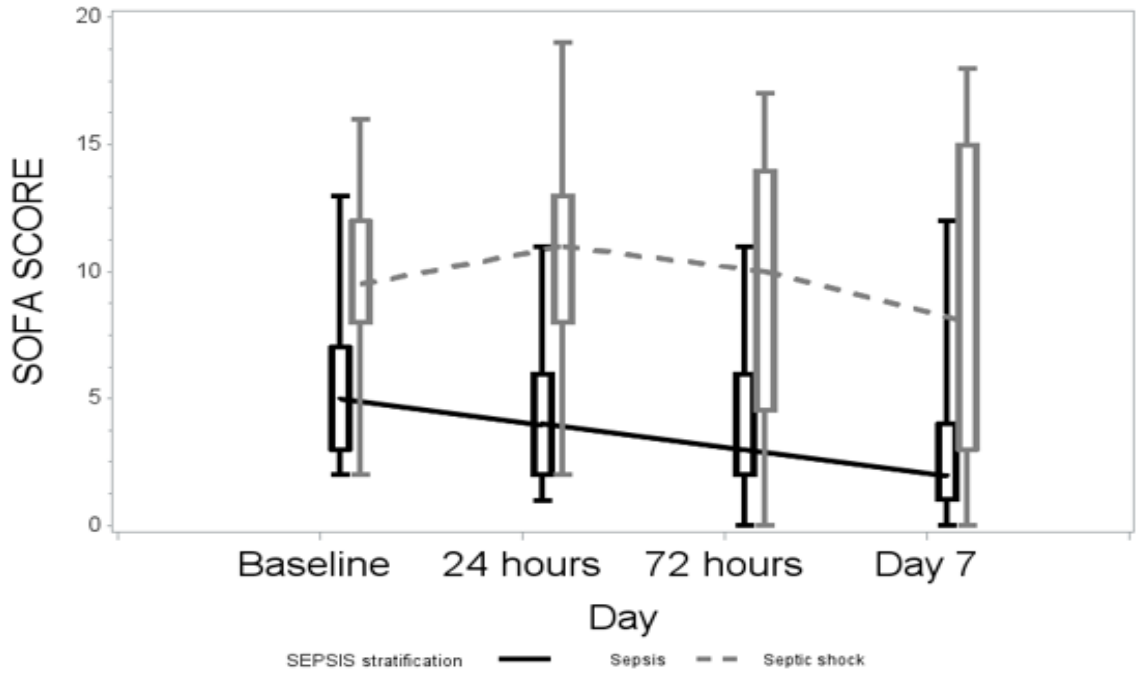


Figure 10. SOFA score values during study in two severity groups

Black line: sepsis group; black dotted line: septic shock group. The vertical left side of the figure shows values of SOFA score. The lower horizontal line shows values of SOFA score at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

Figure 10 shows differences in SOFA score values between two severity groups: sepsis patients and septic shock patients. SOFA score values were significantly higher in patients with septic shock compared to those with sepsis.

Figure 11 presents differences in APACHE II score between two severity groups throughout the study.

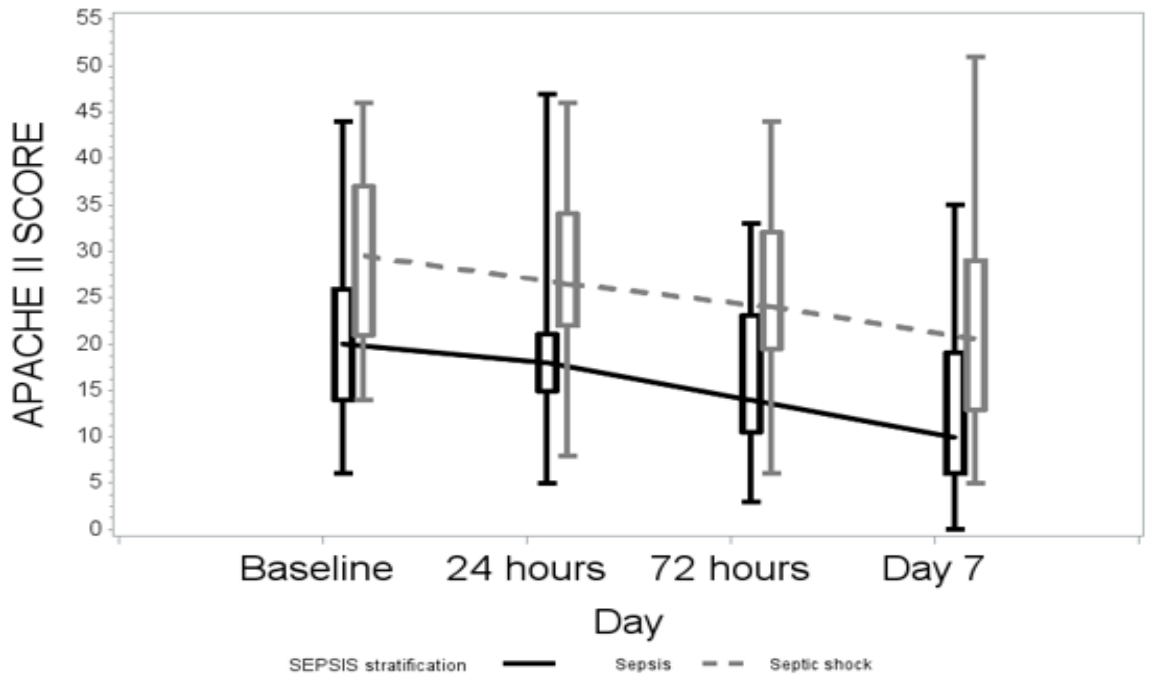


Figure 11. APACHE II score values during study in two severity groups

Black line: sepsis group; black dotted line: septic shock group. The vertical left side of the figure shows values of APACHE II score. The lower horizontal line shows values of APACHE II score at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

Longitudinal analysis of APACHE II and SOFA scores showed that dynamics of their values differed significantly between two severity groups.

Generalized mixed effects model was used to test the changes in APACHE II and SOFA scores during the illness and to estimate the difference between two severity groups after an adjustment for baseline values. Figures 10 and 11 show that scores decreased significantly during the trial (<0.0001), and that severity groups differed significantly. Adjustment was done because baseline score values have a strong impact on subsequent values.

Higher APACHE II values and slower decrease were associated with severity of illness (p=0.0065). The same was found for SOFA score (p=0.0006).

5.8. Associations of CRP and PCT values with disease outcome and severity of sepsis

We analysed dynamics of inflammatory biomarkers to estimate their association with sepsis outcome and sepsis severity (Tables 18 and 19; Figures 12 and 13).

Tables 18 and 19 show the values of CRP and PCT over the course of the disease in two outcome groups, survivors and non-survivors.

Table 18. CRP values in two outcome groups

	Survivors	***Non-survivors***	***Overall***		***Survivors***	***Non-survivors***	***Overall***
	CRP on admission				***CRP after 72 hours***		
N	68	32	100	**N**	66	18	84
Median	197.0	199.0	197.0	**Median**	94.6	138.3	100.1
25th percentile	121.3	141.5	128.0	**25th percentile**	65.8	82.6	71.1
75th percentile	250.0	278.8	259.5	**75th percentile**	146.4	166.7	151.1
	CRP after 24 hours				***CRP on Day 7***		
N	67	25	92	**N**	63	12	75
Median	182.3	209.7	191.5	**Median**	44.2	83.9	56.8
25th percentile	110.0	128.5	112.7	**25th percentile**	18.0	65.8	21.8
75th percentile	226.2	317.4	229.8	**75th percentile**	83.0	154.9	90.3

Abbreviations: CRP-C-reactive protein

There were no significant differences in CRP and PCT values between survivors and non-survivors.

Increased CRP values were not associated with poor outcome. Multivariate analysis showed insignificant impact on patients' outcome after an adjustment for timing and initial CRP values (p=0.2799).

A multivariate analysis of initial biomarkers values on patients' outcome is shown in Table 20.

Table 19. PCT values in two outcome groups

	Survivors	*Non-survivors*	*Overall*		*Survivors*	*Non-survivors*	*Overall*
	PCT on admission				***PCT after 72 hours***		
N	68	32	100	**N**	66	18	84
Median	8.5	12.3	9.0	**Median**	2.0	2.7	2.2
25th percentile	1.7	4.3	2.9	**25th percentile**	0.7	1.2	0.8
75th percentile	36.5	22.5	29.9	**75th percentile**	7.1	9.2	7.3
	PCT after 24 hours				***PCT on Day 7***		
N	67	25	92	**N**	63	12	75
Median	5.5	6.5	6.1	**Median**	0.5	1.5	0.5
25th percentile	1.8	3.0	2.2	**25th percentile**	0.2	0.6	0.2
75th percentile	34.8	21.6	24.5	**75th percentile**	1.2	4.1	1.5

Abbreviations: PCT- procalcitonin

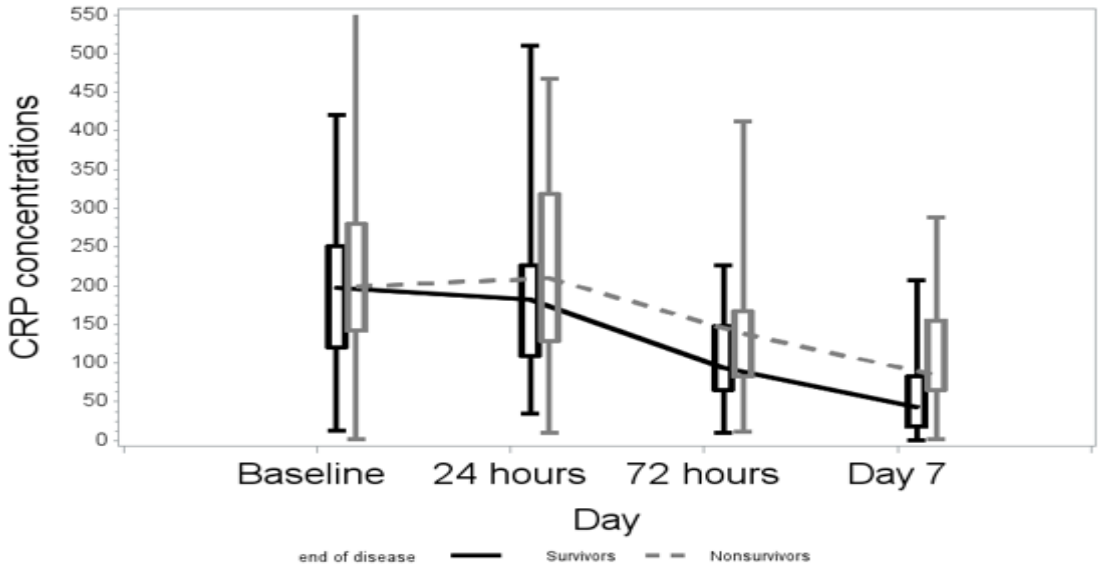


Figure 12. C-reactive protein (CRP) values (mg/L) during study in two outcome groups

Black line: survivors; black dotted line: non-survivors. The vertical left side of the figure shows values of CRP (mg/ml). The lower horizontal line shows values of CRP at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

Figure 13. Procalcitonin (PCT) values (ng/ml) during study in two outcome groups

Black line: survivors; black dotted line: non-survivors. The vertical left side of the figure shows values of PCT. The lower horizontal line shows values of PCT at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

No significant differences were observed in procalcitonin levels between two outcome groups.

Because of a number of procalcitonin missing values, generalized linear mixed effects model was not appropriate due to the lack of convergence.

Finally, multivariate logistic regression analysis was performed to test the association of increased biomarkers with outcome. The model fitted well (Hosmer-Lemeshow test p=0.6226, with satisfactory explanatory value c=0.675).

Only presepsin values on admission were significantly associated with death. Presepsin had a better prognostic value than other tested sepsis biomarkers.

Table 20. Multivariate logistic regression analysis of initial biomarkers values on patients' outcome

Biomarker	***OR***	***95% Wald confidence limits***	
Presepsin on admission	1.011	1.002	1.020
PCT on admission	0.986	0.970	1.003
CRP on admission	1.001	0.998	1.005

Abbreviations: PCT-procalcitonin, CRP-C-reactive protein, OR-odds ratio

Tables 21 and 22 show CRP and PCT values in two severity groups, sepsis and septic shock groups.

Table 21. CRP values (mg/L) during study in two severity groups

	Sepsis	*Septic shock*	*Overall*		*Sepsis*	*Septic shock*	*Overall*
	CRP on admission				***CRP after 72 hours***		
N	66	34	100	**N**	60	24	84
Median	196.9	198.5	197.0	**Median**	111.0	89.6	100.1
25th percentile	125.6	138.5	128.0	**25th percentile**	73.7	69.1	71.1
75th percentile	252.2	264.5	259.5	**75th percentile**	151.1	152.7	151.1
	CRP after 24 hours				***CRP on Day 7***		
N	62	30	92	**N**	57	18	75
Median	180.3	196.1	191.5	**Median**	58.9	54.3	56.8
25th percentile	110.0	128.5	112.7	**25th percentile**	20.9	26.1	21.8
75th percentile	226.8	250.4	229.8	**75th percentile**	83.0	116.5	90.3

Abbreviations: CRP-C-reactive protein

Table 22. PCT values (ng/ml) during study in two severity groups

	Sepsis	*Septic shock*	*Overall*		*Sepsis*	*Septic shock*	*Overall*
	PCT on admission				***PCT after 72 hours***		
N	66	34	100	**N**	60	24	84
Median	6.0	15.6	9.0	**Median**	1.6	2.9	2.2
25th percentile	2.3	5.2	2.9	**25th percentile**	0.7	1.0	0.8
75th percentile	21.9	48.8	29.9	**75th percentile**	6.4	15.9	7.3
	PCT after 24 hours				***PCT on Day 7***		
N	62	30	92	**N**	57	18	75
Median	4.4	11.2	6.1	**Median**	0.5	0.8	0.5
25th percentile	1.9	3.6	2.2	**25th percentile**	0.2	0.4	0.2
75th percentile	18.8	63.4	24.5	**75th percentile**	1.1	3.2	1.5

Abbreviations: PCT- procalcitonin

5.9. Associations of presepsin values with therapy failure

We analysed the associations of presepsin values during the study with therapy failure (Table 23, Figure 14).

We defined therapy failure as a change in antibiotic therapy over the course of the disease. Fever >37.9°C and MAP <65 mmHg, 72 hours after initiation of antibiotic therapy, were used as clinical parameters based on which antibiotic therapy was changed.

Generalized linear mixed effects model procedure showed that presepsin levels were associated with initial levels ($p<0.0001$) and changed significantly in the first seven days ($p<0.0001$), but were not associated with antibiotic change (therapeutic failure) ($p=0.9302$).

We didn't found any association of presepsin levels during study with antibiotc failure, except the association of intial presepsin levels with antibiotic change.

Table 23. Associations of presepsin values (ng/ml) with therapy failure

	Success	*Failure*	*Overall*		*Success*	*Failure*	*Overall*
	Presepsin on admission				***Presepsin after 72 hours***		
N	61	39	300	**N**	49	35	252
Median	103.3	114.4	106.9	**Median**	32.4	65.7	47.3
25th percentile	29.3	88.9	43.6	**25th percentile**	8.2	12.1	9.7
75th percentile	125.9	155.1	140.8	**75th percentile**	111.1	143.0	118.4
	Presepsin after 24 hours				***Presepsin on Day 7***		
N	53	39	276	**N**	46	29	225
Median	98.7	115.5	105.4	**Median**	11.3	16.4	13.2
25th percentile	23.7	52.0	33.9	**25th percentile**	3.6	4.4	3.9
75th percentile	124.6	152.9	132.0	**75th percentile**	36.7	48.5	43.4

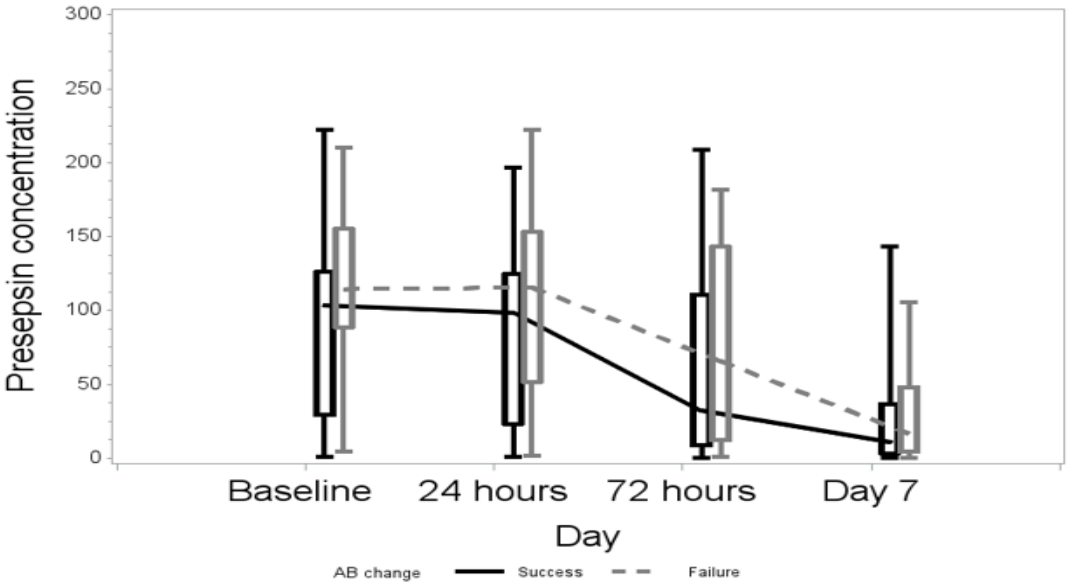


Figure 14. Concentrations of presepsin (ng/ml) in two therapy outcome groups

Black line: success of antibiotic therapy; black dotted line: failure of antibiotic therapy. The vertical left side of the figure shows values of presepsin (ng/ml). The lower horizontal line shows values of presepsin at four time points: on admission, after 24 hours, after 72 hours, and on Day 7.

5.10. Associations of sepsis biomarkers with sepsis outcome and scoring systems

We performed multivariate analysis of associations of presepsin and SOFA values after an adjustment for day of hospitalization (Table 24).

The results showed that presepsin was strongly associated with SOFA score ($p<0.0001$), procalcitonin less but still significantly ($p=0.004$), whereas CRP was not associated with SOFA score ($p=1.827$), and subsequently with the severity of disease.

Table 24. Multivariate logistic regression analysis of SOFA score values with biomarkers after an adjustment for day of hospitalization

Biomarker	*DAY*	*Estimate*	*Standard error*	*DF*	*t Value*	*Pr > |t|*
Intercept		1.2905	0.08667	99	14.89	<.0001
Presepsin		0.002667	0.000649	245	4.11	<.0001
Procalcitonin		0.002392	0.000822	245	2.91	0.0040
CRP		0.000431	0.000322	245	1.34	0.1827
DAY	**1**	0.09219	0.08901	245	1.04	0.3013
DAY	**2**	0.08452	0.08607	245	0.98	0.3271
DAY	**3**	0.1076	0.07777	245	1.38	0.1677
DAY	**4**	0	.	.	.	.

However, presepsin, procalcitonin and CRP correlated with APACHE II score (Table 25), which is less sensitive in predicting multiple organ failure than SOFA score.

Presepsin was a better predictor of multiple organ dysfunction syndrome compared to other tested sepsis biomarkers.

Table 25. Multivariate analysis of associations of APACHE II score values with biomarkers after an adjustment for day of hospitalization

Biomarker	*DAY*	*Estimate*	*Standard error*	*DF*	*t Value*	*Pr > |t|*
Intercept		2.6417	0.05477	99	48.23	<.0001
Presepsin		0.001378	0.000378	245	3.64	0.0003
Procalcitonin		0.001294	0.000516	245	2.51	0.0127
CRP		0.000507	0.000184	245	2.76	0.0062
DAY	**1**	0.1528	0.04978	245	3.07	0.0024
DAY	**2**	0.1291	0.04737	245	2.72	0.0069
DAY	**3**	0.1034	0.04195	245	2.47	0.0144
DAY	**4**	0	.	.	.	.

5.11. Association of presepsin with procalcitonin and C-reactive protein

We analysed the association of presepsin concentrations with the one of procalcitonin (PCT) and C-reactive protein (CRP) (Table 26).

Table 26. Association of presepsin with procalcitonin (PCT) and C-reactive protein (CRP)

Biomarker	*Num DF*	*Den DF*	*F Value*	*Pr > F*
Procalcitonin	1	249	172.56	<.0001
CRP	1	249	1189.88	<.0001

Presepsin correlated significantly with procalcitonin and CRP values.

6. DISCUSSIONS

Sepsis is a life-threatening condition, which has remained among the leading causes of death in critically ill patients (7), despite the appropriateness of fluid resuscitation, use of supportive therapy and timely administered antimicrobial therapy. Identifying septic patients has always been a great challenge to clinicians. Early recognition and timely treatment of patients with sepsis is crucial for better disease outcome (80). Blood cultures have remained a gold standard for sepsis diagnosis, but they are often negative and it takes at least 3 to 5 days to obtain their results. Consequently, researchers have been evaluating the diagnostic value and accuracy of different sepsis biomarkers, and the possibility of their use in early identification of sepsis. Different sepsis biomarkers have been proposed to assess the risk of sepsis in critically ill patients. There are a few studies in which the correlation between sepsis biomarkers and positivity of blood cultures was evaluated, and first results are promising. Presepsin and procalcitonin have shown good correlation with blood culture positive results (72, 81).

Some of the biomarkers are already in use as additional laboratory diagnostic criteria for sepsis, such as serum C-reactive protein and procalcitonin levels 2 standard deviation above normal values (2).

Soluble CD14 subtype (sCD14-ST) or presepsin, first described 15 years ago (9), although still in an evaluation phase for its diagnostic accuracy, appears to be a promising sepsis biomarker. A large number of studies have shown its capacity for distinguishing non-infectious SIRS from sepsis. Its detection in the first hours of establishment of sepsis makes this biomarker greatly useful for early identification of septic patients, and as well, for timely administration of antibiotic therapy. Its capability to rise at different levels between survivors and non-survivors is another advantage of this biomarker that can be used for disease prognostication.

In the present study, we evaluated patients' demographic and clinical features, and we also followed routine laboratory findings, and measured C-reactive protein, procalcitonin and presepsin levels. The aim of the study was to assess diagnostic accuracy and prognostic value of presepsin in septic patients, and compare its diagnostic and prognostic value with the one of procalcitonin and C-reactive protein. Also, the aim was to evaluate the association of presepsin with scoring systems, SOFA score and APACHE II score. Additionally, we aimed to evaluate the

associations of presepsin values with antibiotic therapy failure in septic patients, in order to define its possible use in therapy guidance.

Sepsis is a condition that mostly affects the elderly, probably due to the accompanying comorbidities that *per se* increase the risk for infection, as well as the impaired age-related immunologic defense in older age. Most studies have shown that more than half of patients with sepsis are ≥60 years old (4, 7, 82-84). The same was found in the present study, with 53% of patients ≥65 years old. Median patients' age found in this study is comparable to the European SOAP study, the EPISEPSIS study of severe sepsis in French ICUs, and to the Norwegian prospective study on community acquired sepsis (4, 8, 82).

Most studies have reported than males are more likely to develop sepsis than females, even though the reasons are unclear (4, 8, 10, 29, 82, 84, 85). We found a small predominance of females in our study (52%). A slight predominance of female patients with severe sepsis was also found by Angus et al., in a large observational cohort study on 192,980 severe sepsis patients (7). Most studies have reported that males are more likely to die from sepsis than females (7, 29, 82-84). In our study, we also found differences in mortality between genders. The number of male patients who died from sepsis was greater compared to female patients.

In the present study, the overall ICU mortality was 32%. Comparable to our results, Gašparović et al., in a Croatian national pilot study, on 5,293 patients with sepsis syndrome, found an overall mortality of 29.1% for ICU treated septic patients (28). Similarly, Vincent et al. in the European multicenter observational SOAP study, reported ICU mortality from severe sepsis to be 32.2% (4). In our study, mortality from sepsis and septic shock was 15.2% and 64.7%, respectively. Degoricija et al. (29), in a 6-year retro-prospective study in Croatian medical ICUs, reported mortality from severe sepsis to be 17.0%, which is in line with our results. In the European SOAP study (4), results similar to ours for mortality from sepsis were recorded in Scandinavian ICUs, 14.0% vs. 15.2%. Compared to Degoricija et al., mortality from septic shock was lower in our study, 72.1% vs. 64.7%, but higher than that found by Vincent et al. in the European SOAP study, in which mortality from septic shock was reported to be 54.1%.

Respiratory tract was the most common site of infection. Our findings are consistent with results of other studies (4, 7, 28, 71, 82, 84-86). We recorded the highest mortality among patients with respiratory site of infection and the lowest among patients with genitourinary site of infection. Higher mortality in patients with respiratory site of infection was also noted by Degoricija et al. (29). In our study, respiratory infections, followed by genitourinary infections, intra-abdominal and

skin and soft tissue infections, were the four most common sites of infection. Those four sites of infections accounted for more than 80% of all infection sources.

Different studies have determined that more than 50% of patients with sepsis have at least one risk factor (4, 7, 8, 10, 29, 82, 84-86). This is in line with our findings, with 65.0% of enrolled septic patients having at least one risk factor. Diabetes is frequently reported as a risk factor for developing sepsis, especially sepsis that arises from genitourinary tract. In our study, diabetes was found as the most common comorbidity (45.0%). In a retro-prospective Croatian sepsis study, the same percentage of septic patients having diabetes as a risk factor, was found (29). There were no significant differences regarding chronic accompanying comorbidities between two outcome groups (survivors and non-survivors), except for chronic obstructive pulmonary disease. We found COPD as a risk factor associated with poor outcome (p=0.028). That COPD has an impact on disease outcome in septic patients, was also reported by Degoricija et al. (29).

In the elderly and critically ill patients, body temperature higher than 38°C is less common than in young adults. In a large retrospective study on 1,692 patients with *Staphylococcus aureus* bacteremia, Yahav et al. reported that 37.5% of patients ≥65 years presented with normal body temperature (87). In our study, more than half of patients were ≥65 years old, and presented with normal body temperature on admission in more than 50% of cases. Altered mentation was often seen in our patients. Almost half of them had a GCS ≤13. Mean overall GCS in our patients is comparable to the one found by Degoricija et al. (29).

Acute kidney injury (AKI) is seen in over half of ICU treated patients, irrespective of its etiology. In a large international multicenter cross-sectional study on acute kidney injury on 1,802 patients, acute kidney injury on the first day of ICU treatment was reported in 57.3% of patients (88). The same study used KDIGO criteria for acute kidney injury classification. They reported KDIGO stage 1, stage 2, and stage 3 AKI to be present in 18.4%, 8.9%, and 30.0%, respectively. Sepsis was the most common reason for ICU admission, in which AKI was recorded in 40.7% of patients. A Finnish prospective observational multicenter study on 935 patients with severe sepsis reported acute kidney injury to be present in 53.2% of ICU patients (89). KDIGO stage 1, stage 2, and stage 3 acute kidney injury in that study was found in 21.1%, 10.6%, and 21.5%, respectively. In our study, we found kidney injury in 45.0% of our patients. KDIGO stage 1, stage 2, and stage 3 acute kidney injury on admission was recorded in 17.0%, 16.0%, and 12.0%, respectively. In comparison to Finnish study on incidence of acute kidney injury in septic patients, we found smaller percentage of stage 3 acute kidney injury patients. We assume that the discrepancy may

be due to differences in sepsis definitions and percentage of septic shock patients included in both studies. First, differently to the Finnish study, we used new Sepsis-3 definitions for disease stratification of our septic patients. Second, in our study the percentage of septic shock patients was lower compared to the number of septic shock patients included in the Finnish study, 36.0% vs. 59.6%, respectively. Most studies have reported that there is a correlation between sepsis severity and degree of kidney injury, and the most affected patients are precisely those with septic shock.

Liver is not so often affected in septic patients in the very first days of sepsis establishment, but its degree of injury is related to disease outcome. There are no defined criteria for sepsis-associated liver dysfunction. Various studies have used different definitions for liver injury and failure. In retrospective studies, liver dysfunction is sometimes described using medical coding systems such as International Classification of Disease, Ninth Revision Clinical Modification (ICD 9 CM). Therefore, we could not use those studies for comparison.

The national prospective multicenter study on severe sepsis epidemiology in French ICUs, “the EPISEPSIS study” on 546 severe sepsis documented patients, reported liver dysfunction and failure to be 46.6% and 6.3%, respectively (8). The Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis trial, “the PROWESS trial”, in a study on 1,728 adults with severe sepsis, reported liver dysfunction in 35.6% of patients, whereas liver failure was found in 2.8% of severe sepsis patients (90). In a Japanese multicenter prospective study on 1,104 patients with severe sepsis, Fujishima et al. (91) defined liver dysfunction as total bilirubin level >2 mg/dl. The same level complies with hepatic SOFA score of 2 points. They reported liver dysfunction in 16.7% of their severe sepsis patients. Phua et al. (92) in a prospective cohort study on 1,285 severe sepsis patients, using the same total bilirubin level as Fujishima et al., reported liver dysfunction in 19.3% of enrolled septic patients. In our study, liver dysfunction was recorded in 17.0% of patients. Our results are very similar to those found by Fujishima et al. and Phua et al., but largely differ from results found in the EPISEPSIS and the PROWESS study. We used hepatic SOFA score of 2 points as a criterion for defining liver dysfunction, whereas in the EPISEPSIS study and the PROWESS study, liver dysfunction was defined as SOFA score of 1 or 2 points. We can assume that in our study the number of patients with liver dysfunction would also be greater if, in addition to SOFA score of 2 points, we also used SOFA score of 1 point when defining liver dysfunction, as was done in the two above mentioned studies. We recorded liver failure in 4.0% of our septic patients. Our results are in the range between the results found by the PROWESS study and the EPISEPSIS study, 2.8% and 6.3%, respectively.

In various studies, coagulation disorders have been defined differently. Usually, a decreased platelet count, set at different values (platelet count <150,000/μl, <100,000/μl, or <50,000/μl) is used as a criterion for defining coagulation disorders. Some authors have also used prolonged International Normalized Ratio (INR) or Partial Thromboplastin Time (PTT) as criteria for defining coagulation disorders in septic patients. In order to determine the frequency of coagulation disorders in our patients, we used both, the decreased platelet count and prolonged INR or PTT. In the present study, 42.0% of patients had decreased platelet count, or prolonged INR or PTT, or a combination of both parameters (PLT <100,000/μl + INR >1.5 or PTT >80"). We recorded thrombocytopenia, prolonged INR or PTT, and the combination of thrombocytopenia with prolonged INR or PTT, in 29.0%, 19.0%, and 6.0%, respectively. Moderate thrombocytopenia, defined as platelet count <100,000, is often seen in ICU septic patients. In the EPISEPSIS study, coagulation disorders were found in 45.7%, whereas the PROWESS study reported coagulation disorders to be present in 34.7% of septic patients on admission (8, 90). Both studies used SOFA score of 1 or 2 points for defining hematological dysfunction. In the Japanese multicenter prospective study by Fujishima et al. (91), coagulation disorders were defined as decreased platelet count <100,000/μl and prolonged INR >1.5 or PTT >60". They found coagulation disorders in 43.8% of septic patients. Those results are very similar to ours. In an Asian prospective cohort study, Phua et al. (92) found thrombocytopenia, defined as platelet count <100,000/μl, in 25.3% of severe sepsis patients on ICU admission, and coagulopathy defined as INR >1.5 or PTT >60", in 23.3% of patients. Our results for coagulation disorders are comparable to those found by Fujishima et al. and Phua et al., and they are also comparable to and stand in the range between the results found in the PROWESS and the EPISEPSIS study.

In a large 12-year observational retrospective study on 101,064 severe sepsis patients treated in ICUs of Australia and New Zealand, Kaukonen et al. (93) reported respiratory failure and mechanical ventilation support in 45.0% of included patients. In the SOAP study, conducted by Vincent et al. (4), the percentage of septic patients that needed mechanical ventilation support was reported even higher–80.0%. We recorded the need for mechanical ventilation support in 20.0% of our septic patients. Degoricija et al., in a Croatian retro-prospective study of 314 septic patients treated in medical ICUs, reported that 19.4% of septic patients needed respiratory support by mechanical ventilation. Our results differ from other studies with a much higher number of enrolled patients, but are in line with those found by Degoricija et al. (29).

Blood culture results in our study were positive in 26% of patients, and 35% of all included patients had microbiologically documented infection, which is a lower percentage compared to most

epidemiological studies (4, 8, 85). We found lower percentage of microbiologically documented infections for several reasons. First, almost half of the patients included in our study were treated with antibiotics prior to hospitalization. Second, we included all consecutive sepsis suspected patients, and did not select to include only patients with microbiologically confirmed infection, as was done in the EPISEPSIS study or in the epidemiological study by Alberti et al. (8, 85). Third, in all other septic patients with no microbiologically documented infection, we confirmed the infection by imaging techniques (ultrasound, radiography, computed tomography), or clinically, by evident signs of infection at the infection site (inflammatory signs at the central venous catheter site, or inflammatory signs in patients with soft tissue infections, etc.). Fourth, knowing that the positivity of blood culture results is closely associated with previous antimicrobial treatment, we can assume that even when the initial antibiotic treatment was not appropriate it had an impact (inhibited) on the growth of microorganisms in cultivation grounds. Although we had a low rate of positive blood culture results, similarly to previously reported results (4, 8), we also found a predominance of Gram-positive bacteria in blood culture isolates compared to Gram-negative bacteria, 53.8% vs. 46.2%, respectively. *Staphylococcus aureus* and *Coagulase negative Staphylococcus* were the most common Gram-positive agents isolated from blood cultures, whereas among Gram-negative microorganisms, *Escherichia coli* was most frequently isolated. We found polymicrobial blood culture-based etiology in 3.8% of our patients with blood culture positive results. Since, *Coagulase negative staphylococci* were isolated from critically ill ICU admitted patients that often undergo invasive procedures such as placement of central venous catheter, we can speculate that *Cogulase negative staphylococci* were the causative agent of sepsis, although contamination of blood samples can not be excluded. In critically ill immunocompromised patients, *Coagulase negative staphylococci* are often recorded as causative agents of sepsis (101). A polymicrobial culture-based etiology of sepsis was recorded in 20.0% of all patients with microbiologically documented infection, which is in line with the European SOAP study. The European SOAP Study Group (4) reported suspected clinical infection with identification of pathogens in 38.6% of ICU treated septic patients, which is similar to our results. They also reported Gram-positive organisms to be more frequently isolated. Polymicrobial etiology of sepsis in the European SOAP study is also similar to the one found in our study, 18% vs. 20%, respectively. The French EPISEPSIS Study (8) reported clinically and microbiologically documented infection in 62.1% of septic patients. Similarly to our results, they found a predominance of Gram-positive organisms compared to Gram-negative bacilli. Alberti et al. (85), in a multicenter prospective international cohort study on 3,239 ICU treated septic patients from overall 3,946 patients included in the study, reported a percentage of bloodstream

documented infections very similar to the one found in our study, 21.8% vs. 26%, respectively. They reported microbiologically documented infection from sites other than blood, in 78.2% of septic patients. Differently to our results, they reported Gram-negative organisms to be more frequently isolated from blood or site cultures.

When evaluating differences in biomarkers concentrations between the two outcome groups, we found significantly higher presepsin concentrations in non-survivors compared to survivors at baseline and at every subsequent measurement, as well as significantly higher concentrations of presepsin in septic shock group compared to sepsis group. We did not find significant differences in procalcitonin or C-reactive protein concentrations between non-survivors and survivors on admission or at any other subsequent measurement. Disease outcome was significantly correlated with the level of presepsin and day of measurement. We found a strong association of initial presepsin concentrations with disease outcome. The higher the initial concentration of presepsin, the more adverse the disease outcome. Differences in initial presepsin concentrations between two outcome groups, and its relation with mortality from sepsis, was previously described in other studies (68, 70-73, 94-96). Our results confirmed their findings of higher presepsin concentrations in non-survivors compared to survivors and the in-hospital mortality predictive ability of initial presepsin concentrations in septic patients.

Similarly to our results, Liu et al. (68), in a prospective study on 859 patients presenting to the emergency department, found significantly higher levels of presepsin in non-survivors compared to survivors. They also found initial presepsin concentrations to be an independent predictor of a 28-day mortality, but the same was not observed for procalcitonin. Differently to our study, they used Sepsis-2 definitions criteria, enrolled healthy subjects in the study, and measured presepsin concentrations on admission only. Spanuth et al. (95), in a study on 146 septic patients presenting to emergency room, measured subsequently presepsin, procalcitonin, C-reactive protein and IL-6 levels on admission, after 24, and 72 hours. Similarly to our results, they found significantly higher levels of presepsin in non-survivors compared to survivors, and no significant differences in procalcitonin, IL-6, and CRP levels between two outcome groups, on admission. Presepsin showed the highest capacity to predict a 30-day mortality, compared to other biomarkers. The study was published in 2011, so criteria used for sepsis definitions were different from ours. Klouche et al. (94), in an observational prospective study on 144 patients admitted to two different ICUs in France, evaluated presepsin, procalcitonin, and high-sensitivity C-reactive protein (hsCRP) concentrations on admission in patients with sepsis, septic shock, and community acquired pneumonia. Sepsis-2 definitions criteria were used for defining the severity of sepsis.

They found significantly higher levels of presepsin between non-survivors and survivors, and no significant differences in hsCRP levels between non-survivors and survivors, which is in accordance with our results. Differently to our results, they also found significantly higher procalcitonin levels in patients who died compared to those who survived, even though presepsin concentrations on admission predicted better the ICU mortality in septic patients compared to procalcitonin. In a more recent retrospective study on 157 septic patients, classified retrospectively into sepsis and septic shock groups, Kim et al. (96) evaluated the ability of several new sepsis biomarkers to predict mortality – that of presepsin, procalcitonin, galectin-3, and soluble suppression of tumorigenicity 2 (sST2). For all tested biomarkers, initial levels were significantly higher in non-survivors compared to survivors, except for procalcitonin. Similarly to our study, Sepsis-3 definitions were applied for sepsis severity stratification. Presepsin concentrations on admission were significantly associated with disease outcome, whereas procalcitonin concentrations were not, which is in line with our results.

In the present study, presepsin concentrations showed a different trend over time in non-survivors compared to survivors. While in non-survivors presepsin concentrations remained high during the first week of measurement, in survivors presepsin concentrations decreased gradually. As for procalcitonin levels, they decreased in a similar way in both outcome groups. Masson et al. (72), in a large multicenter randomized Albumin Italian Outcome Sepsis trial (ALBIOS trial), in a subgroup of 997 severe sepsis patients in which biomarkers were measured, found presepsin concentrations significantly different between non-survivors and survivors. Measurements of presepsin concentrations were done on Day 1, 2, and 7 after enrolment. Similarly to our results, they found the initial presepsin concentration to be strongly associated with mortality, as well as higher or increasing levels of presepsin over time in non-survivors, and decreasing trend of presepsin in survivors. In another study by Masson et al. (73), with a smallest subgroup of patients from the ALBIOS trial, when comparing presepsin to procalcitonin value for mortality prediction, they again found higher initial presepsin concentrations in non-survivors compared to survivors. They also found presepsin to be the only biomarker that was strongly associated with disease outcome. They described a decreasing trend of presepsin concentrations in survivors, and remaining high concentrations of presepsin in non-survivors in all subsequent measurements, whereas procalcitonin levels decreased similarly in both outcome groups, which is in line with our results. They found presepsin to be superior to procalcitonin for mortality prediction in septic patients. Ulla et al. (70), in a multicenter prospective study in emergency departments of two Italian hospitals, included 106 septic patients and 83 non-infectious SIRS controls. They

measured presepsin concentrations on admission, after 24 hours, and after 72 hours. They found significantly higher initial presepsin concentrations in non-survivors compared to survivors. Differently to our study, they found a correlation only of initial presepsin values with poor disease outcome. In a mono-centric prospective study on 116 ICU treated septic patients, Behnes et al. (69) measured the levels of several sepsis biomarkers: presepsin, procalcitonin, C-reactive protein, and IL-6; 24 hours after the onset of sepsis or septic shock, on Day 3 and Day 8 after enrolment, and evaluated the diagnostic and prognostic capacity of the measured biomarkers. Similarly to our findings, they found significantly higher levels of presepsin in non-survivors compared to survivors on the first measurement and at any subsequent measurement, and no significant differences in procalcitonin or C-reactive protein levels between non-survivors and survivors, thus showing the superiority of presepsin compared to procalcitonin and C-reactive protein in mortality prediction. They also found significantly higher levels of IL-6 on the first measurement, with a similarly decreasing trend over time in both outcome groups.

Our results support the prognostic role of presepsin in in-hospital mortality prediction. The decreasing trend of presepsin in surviving patients may be due to the appropriateness of antibiotic therapy, recovered renal function and appropriate clearance of presepsin. Studies have shown that kidneys are an important organ for presepsin clearance (74), so we can speculate that in patients with appropriate antibiotic therapy and more rapidly recovered kidney function, presepsin was cleared earlier than in those with severely impaired renal function and inappropriate antibiotic therapy. The decrease of absolute values of measured sepsis biomarkers, CRP, PCT and presepsin, in non-surviving patients, may be due to the lack of stimulation of their production or due to their increased elimination. It is already demonstrated that presepsin production is related to bacterial phagocytosis (62), so we can speculate that in non-surviving patients, even when the bacterial infection is defeated, patients are dying from multiple organ dysfunction caused by the powerful inflammatory response.

When constructing the ROC curve, for testing the importance of the impact of initial presepsin values on disease outcome, we found a threshold value of 90 ng/ml for discriminating favorable from unfavorable outcome. At the same threshold value, presepsin initial concentrations showed a high specificity but low sensitivity. We could not compare the threshold value for predicting in-hospital mortality with the one found in other studies for several reasons. First, we could not find any study in which presepsin measurements were done using the same technique of measurement that we performed for measuring presepsin levels in our study. In most studies, presepsin levels have been measured using a compact, automated immunoanalyzer,

PATHFAST, based on a chemiluminescent enzyme immunoassay, with presepsin concentrations and presepsin threshold values mainly reported in picograms per milliliter. Second, we did not find any study in which, by using the same technique of measurement, septic patients were classified according to Sepsis-3 criteria. Third, there are a few studies in which presepsin concentrations were measured with the ELISA technique, but even in those studies, the impact of presepsin concentration on mortality prediction was not evaluated.

When evaluating differences in presepsin concentrations between two severity groups, we found significantly higher presepsin concentrations in septic shock group compared to sepsis group patients. Our study revealed that presepsin concentrations had a good capacity for distinguishing disease severity. Serum presepsin concentration on admission reflected the severity of disease. The higher the presepsin concentration on admission in septic patients, and the slower the decrease of presepsin concentrations, the more severe the clinical presentation and the greater the possibility of having septic shock. Severity of disease was strongly associated with presepsin concentrations and day of measurement. Presepsin concentrations significantly differed between two severity groups on admission and in the first 72 hours. We did not find significant differences between two severity groups on Day 7. This may be due to the fact that more than half of patients with septic shock died before Day 7. In our study, we did not find a correlation between procalcitonin and C-reactive protein with severity of disease. Procalcitonin and C-reactive protein levels did not differ significantly between two severity groups.

Comparison of our results for association of sepsis biomarkers with severity of disease to those found in other studies was difficult because most studies, in which sepsis biomarkers capability for discriminating sepsis severity was evaluated, used either old sepsis definitions or used, in addition to sepsis group patients, also SIRS patients or healthy controls. However, most studies related to diagnostic accuracy of presepsin reported that presepsin has a good discriminating capacity between sepsis severity groups (68, 71, 72, 94, 96, 97).

In a study with a single presepsin measurement, presepsin ability to discriminate between sepsis severity groups was reported. Similarly to our results, Liu et al. (68) reported significantly higher levels of presepsin in patients with septic shock compared to those with sepsis or severe sepsis. They also found significantly higher levels of procalcitonin between sepsis severity groups, which was not observed in our study. Behnes et al. (71), in multiple measurements of sepsis biomarkers, reported a good ability of presepsin for distinguishing severity of sepsis. They found significantly higher levels of presepsin in septic shock patients compared to septic patients. In line with our

results, they found better diagnostic ability of presepsin to distinguish septic shock from sepsis compared to C-reactive protein and procalcitonin. In a subgroup study of the ALBIOS trial, on 997 septic patients in which biomarkers were measured several times during the first week of ICU treatment, Masson et al. (72) found significantly higher concentrations of presepsin in septic shock patients compared to septic patients. Klouche et al. (94) also found significantly higher levels of presepsin in septic shock patients compared to sepsis patients. However, they also found significantly higher levels of procalcitonin between septic shock and sepsis patients. Spanuth et al. (95) found significantly higher levels of presepsin, as well as higher levels of procalcitonin and C-reactive protein in septic shock patients compared to septic patients. Diagnostic accuracy of presepsin was superior to that of procalcitonin and C-reactive protein. In a recently published study, Ali et al. (98) reported sepsis stratification using Sepsis-3 definitions in their study group on 51 patients, but they actually classified patients in SIRS groups with or without sepsis. There was no stratification of patients into the sepsis and septic shock group as recommended by the Sepsis-3 Definitions Consensus Conference, so, we could not compare our results with those found in that study. The same study found presepsin diagnostic and prognostic value to be comparable to that of procalcitonin. In another recently published study, a prospective observational single-centre study on 130 septic patients and 70 non-septic patients with documented infection, de Guadiana Romualdo et al. (99), used Sepsis-3 Definitions criteria to compare diagnostic accuracy of presepsin to that of procalcitonin and C-reactive protein. They found a good diagnostic accuracy of presepsin and procalcitonin in diagnosing sepsis and distinguishing sepsis from septic shock. Both presepsin and procalcitonin showed significantly higher levels on admission in septic shock patients compared to septic patients. The same was not found for C-reactive protein. Yamamoto et al. (100)**,** in a prospective observational study on 91 patients published in 2019, retrospectively classified patients according to Sepsis-3 definitions criteria into three groups: non-sepsis, sepsis, and septic shock groups, and evaluated the diagnostic accuracy of sepsis biomarkers –presepsin, procalcitonin, and C-reactive protein. They found presepsin to have the highest diagnostic accuracy for discriminating non-sepsis from sepsis and septic shock group as well as between sepsis groups, compared to procalcitonin and C-reactive protein. They found significantly higher levels of presepsin and procalcitonin in sepsis and septic shock groups compared to non-sepsis group, as well as significantly higher levels of both biomarkers, presepsin and procalcitonin, in septic shock groups compared to sepsis group. They found no significant differences in C-reactive protein levels between each group.

In our study, the AUC calculated from the ROC curve of presepsin for diagnosing septic shock was 0.703. We found a cut-off value of presepsin of 110 ng/ml for diagnosing septic shock. Patients with presepsin values above 110 ng/ml were more likely to develop septic shock. Sensitivity and specificity found at the same cut-off value were 72.7% and 61.8%, respectively.

The cut-off value of presepsin for sepsis and septic shock found in our study in not comparable to cut-off values found in other studies for reasons mentioned above. No other study used the same technique for measuring presepsin concentrations and had stratified septic patients according to Sepsis-3 new definitions. Different cut-off values of presepsin for diagnosing sepsis have been reported by different studies. However, the sensitivity and specificity of presepsin for sepsis diagnosis found in our study, were comparable to the results of sensitivity and specificity found in other studies.

In a prospective study on 859 patients presenting with sepsis, Liu et al. (68) reported a larger area under the ROC curve for predicting septic shock, in comparison to our results, 0.790 vs. 0.703. They found higher sensitivity and specificity at the set presepsin cut-off value of 550 pg/ml, compared to the one found in our study, 85.7% and 66.8% vs. 72.7% and 61.8%, respectively.

Behnes et al. (71), in their study on 116 patients with severe sepsis and septic shock, found a cut-off value of 700 pg/ml for diagnosing septic shock. The AUC at the set cut-off value was 0.80. They found high sensitivity and specificity at the set cut-off value of presepsin for diagnosing septic shock, 91% and 77%, respectively. In a recent study on 144 sepsis patients classified as severe sepsis and septic shock, Klouche et al. (94) reported the AUC of presepsin for diagnosing severe sepsis and septic shock to be 0.75. They found a cut-off value of 466.5 pg/ml for diagnosing severe sepsis and septic shock. At the same cut-off value, the sensitivity reported was higher than the one found in our study (90% vs. 72.7%), but the specificity was lower (55% vs. 61.8%).

In recently published studies, Ali et al. (98) and de Guadiana Romualdo et al. (99) found similar cut-off values of presepsin for diagnosing sepsis. At the set cut-off values, they found lower sensitivity but higher specificity compared to our results.

Ali et al. (98), in a study on 51 septic patients classified according to Sepsis-3 definitions criteria, reported the AUC of presepsin to diagnose sepsis to be 0.805. At a cut-off value of 907 ng/l, they reported sensitivity and specificity to be 69.7% and 83.3%, respectively.

De Guadiana Romualdo et al. (99), in a study published in 2017 on 200 patients, from which 70 were septic patients, used Sepsis-3 definitions criteria to diagnose sepsis. They found a cut-off value of 849 ng/ml for presepsin to diagnose sepsis. They reported the area under the curve of presepsin to be 0.775, while sensitivity and specificity at the same cut-off value were 67.1% and 80.8%, respectively.

Outcome prediction and disease stratification by inflammatory biomarkers, such as C-reactive protein (CRP) and procalcitonin (PCT), have been widely studied, but the results are still controversial (contradictory, or inconsistent). For example, there have been studies that determined the good capacity of CRP or PCT or both biomarkers, in disease stratification and outcome prediction, whereas other studies showed that CRP and PCT were unable to distinguish between sepsis severity groups, or to predict mortality.

Despite the fact that procalcitonin and C-reactive protein are helpful markers in the diagnosis of sepsis, their concentrations are often increased in a large number of non-sepsis inflammatory conditions, such as myocardial infarction (102), surgery (103), trauma (104), pancreatitis (105), etc. Recently, presepsin values have been investigated in other non-septic conditions, such as pyelonephritis (106), rheumatoid arthritis (107), trauma (108), kidney injury (109), etc. All those studies have shown increased presepsin concentrations in patients with a disease other than sepsis, only when an infection was present, thus showing its specific increase when infectious disease is present.

Interestingly, in our study CRP and PCT did not show any significant correlation with disease severity or disease outcome. We did not find significant differences in CRP levels between patients with sepsis compared to septic shock patients. CRP levels in our study were not significantly correlated with disease outcome. There were no significant differences in CRP levels between survivors and non-survivors. The same was found for PCT. In our study, the included patients had exclusively infectious diseases, and we did not include any SIRS conditions simulating sepsis. This may be due to the fact that our patients were suspected and diagnosed as sepsis patients by a specialist of infectious diseases, as well as treated in medical ICUs of infectious diseases hospitals where the possibility of having non-sepsis SIRS patients is limited. Our study is the first to investigate presepsin levels in patients treated in hospitals for infectious diseases.

When comparing the diagnostic accuracy of presepsin with that of C-reactive protein in the recent studies (99, 100), C-reactive protein was not found to have any discriminating value in relation to

sepsis severity groups, which is in line with our results. However, some studies (66, 71, 95, 99, 100) found procalcitonin to perform a good diagnostic value even though, when compared to presepsin, the latter showed a better diagnostic accuracy.

Regarding the prognostic accuracy of procalcitonin in septic patients, there have been different results and opinions. Although there have been studies which reported significant differences in procalcitonin levels between survivors and non-survivors, its role in mortality prediction has been questioned. In a meta-analysis published in 2015, Liu et al. (110) found higher procalcitonin levels in non-survivors compared to survivors, but no association of initial procalcitonin levels with mortality (110). Other studies have shown that procalcitonin levels increase later during study and initial procalcitonin levels does not have an impact on mortality (70, 73). When comparing the predictive value of different sepsis biomarkers, including presepsin among them, several studies have shown a superiority of other sepsis biomarkers in predicting short- or long-term mortality compared to procalcitonin, which was found to be less prognostic than other sepsis biomarkers (68, 73, 95, 96).

Scoring systems have been widely used in clinical trials to assess severity of organ dysfunction and mortality prediction in patients with sepsis. SOFA score was designed, widely approved and recommended for evaluation of organ dysfunction and severity of disease in patients with sepsis, whereas APACHE II score was designed to predict mortality in critically ill patients.

In our study, both scores, APACHE II score and SOFA score, were significantly associated with patients' outcome as well as with the severity of sepsis. Even though SOFA score is an organ dysfunction evaluating score and not designed to predict mortality, its mortality prediction derives from the degree of organ dysfunction or failure in septic patients.

We found significantly higher APACHE II score on admission in non-survivors compared to survivors, which is in accordance with previously published results (25, 68, 71, 95, 111-113).

High APACHE II score on admission, that remained high during the study and even increased on Day 7, predicted poor outcome. On the other hand, in patients with favorable outcome, we found lower initial APACHE II score, with a decreasing trend over time.

We found similar results regarding SOFA score association with disease outcome and disease severity. Higher initial SOFA score that increased and remained high over time was associated with death. In surviving patients, the initial value of SOFA score was lower, and serial SOFA scores showed a decreasing trend over time. Our results are in accordance with those found in

other studies (26, 29, 73, 94-96, 113-115). We found significant differences in initial, worst, and serial SOFA score values between non-survivors and survivors. Our study confirmed the good capacity of APACHE II and SOFA scores to predict in-hospital mortality. We showed that the higher the initial values of APACHE II and SOFA scores, the worst the outcome. APACHE II and SOFA scores values on admission had a strong impact on disease outcome.

Both scores, APACHE II and SOFA, were strongly associated with disease severity. Their initial, serial, and worst values were higher in septic shock patients than in septic patients. Higher APACHE II in septic shock, in comparison to other severity groups, was also found by other authors (29, 68, 71, 95, 97).

It is expected to find higher SOFA score in patients with septic shock compared to patients with sepsis without shock, knowing that SOFA score calculates not only the number of organs failing but also the severity of organ dysfunction. In the present study, we confirmed the severity of disease prediction by SOFA score. We found significantly higher SOFA score in septic shock patients compared to septic patients without shock on admission and at every subsequent measurement, as well as significantly higher worst SOFA score in septic shock patients compared to septic patients. Differences between SOFA score in patients with septic shock and sepsis were also found in other studies (29, 71, 94, 95).

Liu et al. (68) found APACHE II score to be an independent predictor of a 28-day mortality. They calculated APACHE II score on 859 patients presenting to the emergency department with SIRS criteria. They also found significantly higher APACHE II score in septic shock patients compared to septic patients. Behnes et al. (71), in a study of multiple calculations of APACHE II and SOFA scores, found higher values of both scores in patients with septic shock compared to those with severe sepsis. They also found higher APACHE II and SOFA scores in non-survivors compared to survivors, which is in accordance with our results. Ferreira et al. (114), in a prospective observational cohort study, calculated SOFA score on 253 ICU treated patients, on admission and every 24 hours until discharge. They found a good correlation of initial, highest, and mean SOFA score with ICU mortality. Klouche et al. (94) also found significantly higher values of SOFA score in sepsis non-surviving patients compared to sepsis surviving patients. Qiao et al. (113), in a prospective study on 106 elderly patients, calculated SOFA and APACHE II scores and evaluated their correlation with disease outcome. They found significant differences in SOFA and APACHE II scores between non-survivors and survivors. Both scores could accurately predict mortality in elderly ICU treated patients. Spanuth et al. (95) found significantly higher APACHE II

and SOFA scores in patients with septic shock compared to those with sepsis, as well as higher values of both scores in non-survivors compared to survivors, which is in accordance with our findings.

The present study showed that presepsin concentration was strongly associated with APACHE II score and SOFA score, thus demonstrating the prognostic capacity of presepsin and its association with severity of disease. We found a correlation of initial and subsequent presepsin values with initial and subsequent values of both calculated scores, SOFA and APACHE II score. Presepsin was a better predictor of multiple organ dysfunction and disease outcome compared to CRP and PCT.

We found a significant correlation of presepsin levels with procalcitonin and C-reactive protein levels. Similar results were found in other studies (68, 71, 98).

One of the aims of the present study was also to evaluate association of presepsin concentrations with first-line antibiotic therapy failure. We considered that clinical parameters better reflect the appropriateness of first-line empirical therapy. Therefore, we defined as therapy failure the persistence of fever and/or hypotension in patients with a change in antibiotic therapy in the first 72 hours after enrolment. We evaluated initial and subsequent presepsin concentrations and their association with antibiotic change during the disease course. We found an association of initial presepsin concentrations and antibiotic therapy failure. Glimix procedure showed that only initial presepsin levels were associated with antibiotic change, and presepsin levels significantly changed during the study and were not associated with antibiotic therapy failure. Only initial presepsin concentrations were associated with antibiotic therapy failure or appropriateness. We didn't find association of subsequent presepsin levels with antibiotic change. Our results show that presepsin levels during the disease course doesn't reflect appropriateness or inappropriateness of antibiotic therapy. So, we can speculate that administration of antibiotics affects presepsin levels, regardless of their appropriateness.

Although in a different way from the one used in the present study, Masson et al. (72) also evaluated association of presepsin concentrations with appropriateness of first-line antibiotic therapy. They evaluated dynamics of presepsin concentrations during study in patients with appropriate initial first-line empirical therapy according to blood and site cultures results. They considered antibiotic therapy inappropriate if it was administered after the first day of enrolment, if the initial antibiotic therapy did not cover all isolated pathogens, and if the pathogens were resistant to first-line antibiotic therapy. They found decreasing levels of presepsin over the first 7

days of treatment in patients with appropriate initial empirical antibiotic therapy, and increasing levels in those with inappropriate initial treatment. They noted that monitoring presepsin levels during the first week of treatment could mirror the adequacy of empirical therapy, thus showing the possibility of use of presepsin in antibiotic guidance.

The present study shows some strengths. To our knowledge, it is the first to evaluate the diagnostic and prognostic accuracy of presepsin in septic patients diagnosed by specialists of infectious diseases. Other sepsis-like inflammatory conditions were not included in the study. All patients included and then diagnosed as having non-sepsis infectious or non-infectious diseases were subsequently excluded from the study. All patients included in the study met the Sepsis-3 definitions criteria. Patients enrolled at the first sub period of the study were retrospectively reclassified as sepsis and septic shock patients according to new Sepsis-3 definitions criteria.

Limitations of the present study include the following: first, the study was conducted in two time periods, with half of included patients enrolled in the first subperiod (2015-2016), and the other half in 2018; second, the study was done on a limited number of patients; third, we could not evaluate the impact of presepsin concentrations on appropriateness of antibiotic therapy due to a small number of blood culture positive results. We do not question the diagnosis of sepsis in our patients but we assume that positivity of blood cultures is related to prior antibiotic treatment of enrolled patients. More than half of included patients were previously treated with antibiotics in emergency departments of other hospitals from where they were transferred to infectious diseases clinic.

7. CONCLUSIONS

Presepsin has a good prognostic capacity. Its elevated concentrations in septic patients that do not decrease during illness suggest poor disease outcome. The higher the serum concentration of presepsin, and the slower its decrease over time, the greater the risk of dying. Moreover, the decreasing trend of presepsin concentrations during illness suggests a favorable disease outcome.

Compared to procalcitonin and C-reactive protein, only presepsin showed a prognostic value.

Procalcitonin levels decreased in a similar way in both outcome groups, survivors and non-survivors.

A strong correlation between SOFA score and presepsin concentrations confirms the usefulness of presepsin in sepsis recognition and disease stratification.

Serum presepsin levels reflect the severity of disease. Serum presepsin concentrations were associated significantly with severity of clinical presentation.

Only initial presepsin concentrations were associated with antibiotic therapy failure.

Procalcitonin and C-reactive protein were not valuable markers for discriminating disease severity, and neither for disease outcome.

SOFA and APACHE II scores were found as reliable scores in predicting outcome and disease severity.

SOFA score value on admission has a strong impact on disease outcome.

8. SAŽETAK

Uvod: Sepsa je životno ugrožavajuće stanje sa teškim i vrlo varijabilnim kliničkim manifestacijama, te ostaje značajan uzrok morbiditeta i mortaliteta. Definicija sepse mijenjala se s vremenom. Nedavno, 2016 godine postavljena je nova definicija sepse. Brza dijagnoza sepse može se postaviti na temelju novih biomarkera sepse. Presepsin je novi biomarker sepse, prvi put opisan prije gotovo 15 godina. Cilj ove studije bio je procijeniti dijagnostičku i prognostičku vrijednost presepsina u septičkih bolesnika, kao i usporediti dijagnostičku i prognostičku sposobnost presepsina s drugim biomarkerima (PCT i CRP) I skoring sustavima (APACHE II i SOFA skor).

Metode: Radi se o prospektivnoj opservacijskoj studiji provedenoj u 2 kliničke bolnice za infektivne bolesti, na Kosovu te u Hrvatskoj. U studiju je uključeno 100 pacijenata sa sepsom. Pacijenti su razmješteni u skupine prema ishodu bolesti (preživjeli i umrli) te prema težini bolesti (sepsa sa i bez septičkog šoka). Biomarkeri sepse (presepsin–PSEP, procalcitonin–PCT i C-reaktivni protein–CRP) su mjereni četiri puta tijekom bolesti (kod prijema–T0, nakon 24 sata–T1, nakon 72 sata–T2 i sedmog dana od početka bolesti–T4), te su izračunati skoring sustavi (SOFA i APACHE II skor). Za mjerenje vrijednosti presepsina korištena je "sendvič" ELISA tehnika. Generalizirani linearni mješoviti model korišten je za analiziranje promjena u koncentracijama presepsina tijekom bolesti te procjenu razlika između skupina različitog ishoda bolesti (preživjelih i umrlih), kao i skupina različite težine bolesti (sepsa sa i bez septičkog šoka). Izračunate su ROC krivulje te površine ispod ROC krivulja (AUC) kako bi se procijenio značaj početnih vrijednosti koncentracija presepsina za ishod i težinu bolesti. Temeljem optimalnih graničnih vrijednosti presepsina za razlikovanje između grupa različitog ishoda i težine bolesti, analizom ROC krivulja izračunate su osjetljivost i specifičnost navedenih graničnih vrijednosti. Za ispitivanje povezanosti vrijednosti koncentracija presepsina i vrijednosti skorova za procjenu težine bolesti (SOFA i APACHE II) korištena je multivarijatna analiza. Za sve je testove korištena razina pouzdanosti od 5 posto. Sve su analize provedene koristeći SAS software verziju 9.3 (SAS Institute, Cary, North Carolina, USA).

Rezultati: koncentracije presepsina bila su značajno više u skupini umrlih bolesnika u usporedbi sa preživjelima, kao i u skupini bolesnika sa sepsom i septičkim šokom u usporedbi sa

bolesnicima bez šoka. Razine PCT-a i CRP-a nisu se razlikovale između skupina različitog ishoda bolesti, kao ni skupina različite težine bolesti. Presepsin je bio jedini marker čije su vrijednosti bile povezane sa težinom bolesti i njezinim ishodom. Nađena je jasna korelacija između vrijednosti presepsina i SOFA i APACHE II skorova. Jedino su početne vrijednosti presepsina bile povezane s terapijskim neuspjehom.

Zaključak: naša je studija pokazala kako je presepsin bolji dijagnostički i prognostički marker sepse u usporedbi s drugim ispitivanim biomarkerima (CRP-om i PCT-om). Početne i subsekventne vrijednosti presepsina bile su značajno povezane s težinom i ishodom bolesti, dok isto nije nađeno za CRP i PCT.

Ključne riječi: sepsa, presepsin, prokalcitonin, C- reaktivni protein, težina bolesti, ishod bolesti, SOFA skor, APACHE II skor.

9. ABSTRACT

Background: Sepsis is a life-threatening condition with poor and highly variable clinical manifestations, which has remained a major cause of morbidity and mortality. Over time, definitions of sepsis have changed. Recently, in 2016, new sepsis definitions were set. At present, rapid diagnosis of sepsis is based on new sepsis biomarkers. Presepsin is a new sepsis biomarker, first described almost 15 years ago. The aim of the present study was to evaluate the diagnostic and prognostic value of presepsin in septic patients, as well as to compare the diagnostic and prognostic ability of presepsin with other biomarkers (PCT and CRP), and scoring systems (APACHE II and SOFA score).

Methods: A prospective observational study was conducted in two university hospitals for infectious diseases, in Kosovo and in Croatia. One hundred consecutive septic patients were enrolled in the study. Patients were grouped according to disease outcome (survivors and non-survivors) and disease severity (sepsis, septic shock). Sepsis biomarkers (presepsin–PSEP, procalcitonin–PCT, and C-reactive protein–CRP) were measured at four time points over the course of the disease (on admission–T0, after 24 hours–T1, after 72 hours–T2, and on Day 7–T3), and scoring systems (SOFA score and APACHE II score) were calculated. A sandwich Human presepsin ELISA Kit was used for presepsin measurements. Generalized linear mixed effects model was used to test the changes in presepsin concentrations during the illness and to estimate the difference between two outcome groups (survivors and non-survivors), as well as between two severity groups (sepsis and septic shock). Receiver Operating Characteristic (ROC) curves and areas under the ROC curves (AUCs) were calculated to test the importance of initial presepsin concentrations on sepsis outcome and sepsis severity. Based on optimal cut-off values of presepsin, for discriminating between outcome groups and severity groups, according to ROC curve analysis, the sensitivity and specificity of the found threshold values was calculated. Multivariate analysis was used to test the association of presepsin values and SOFA and APACHE II scores. For all statistical tests, significance was set at an alpha level of 0.05. All analyses were performed using SAS software version 9.3 (SAS Institute, Cary, North Carolina, USA).

Results: Presepsin concentrations were significantly higher in non-survivors compared to survivors, and in septic shock patients compared to patients with sepsis without shock. PCT and CRP levels did not differ between disease outcome groups, and neither between disease severity groups. Presepsin was the only biomarker associated with disease severity and disease outcome. There was a strong correlation between presepsin and SOFA and APACHE II scores. Only initial presepsin concentrations were associated with therapy failure.

Conclusion: This study shows that, compared to PCT and CRP, presepsin is a better diagnostic and prognostic. Initial and subsequent presepsin concentrations were significantly associated with disease severity and disease outcome. The same was not found for PCT and CRP.

Key words: Sepsis, presepsin, procalcitonin, C-reactive protein, disease severity, disease outcome, SOFA score, APACHE II score.

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11. CURRICULUM VITAE

Ajete Aliu-Bejta was born on September 4th, 1977 in Pristina, Kosovo. She has finished her primary school in France, where she lived with her parents. She graduated from the Medical Faculty, University of Pristina in 2005. She completed her residency in infectious diseases at the University Clinical Center of Kosovo, Clinic of Infectious diseases in Pristina, Kosovo, in 2013.
She is currently working as specialist of infectious diseases at the University Clinical Center of Kosovo in Pristina, Clinic of Infectious diseases– Pediatric Department. In 2013, she started working as an external associate at the University of Pristina–Faculty of Medicine, department of infectious diseases. She has actively participated in a number of local, regional and international conferences. She has written two chapters of a university book for infectious diseases („Semundjet infektive“, Univerziteti i Prishtines, 2018), and published an article and several abstracts related to PHD thesis.