EVALUATION OF PROGNOSTIC SIGNIFICANCE FOR BIOCHEMICAL MARKERS OF OXIDATIVE STRESS, ENDOGENOUS INTOXICATION AND VASCULAR REGULATION IN THE DEVELOPMENT OF UNFAVORABLE OUTCOMES IN PATIENTS WITH SEPSIS

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BACKGROUND	Due to the significant infectious complications mortality, the search for prognostic biochemical markers of sepsis development in critically ill patients is relevant.
MATERIAL AND METHODS	The study involved 57 patients; in 40 cases sepsis developed. The control group included 17 patients where sepsis did not develop. The study was performed on day 1–2, 5–7 and 10–12 after admission. The intensity of oxidative stress was assessed by the level of malondialdehyde (MDA) and total antioxidant activity of blood serum (TAA). The endogenous intoxication was assessed by the concentration of medium molecular weight peptides (MMWPs), total and effective albumin concentration (TAC, EAC) in serum. The disturbance of endogenous vascular regulation was assessed by the level of stable metabolites of nitric oxide (NOx) in serum and the concentration of angiotensin converting enzyme (ACE).
RESULTS	It has been found that MDA may be a prognostic index of poor outcome on day 5–7 after admission (relative risk (RR)=1.141, confidence interval (CI) 95% (1.033; 1.259), p=0.09); NOx level may be a predictor of a poor outcome on day 1–2 (RR=1.026, CI 95% (0.999; 1.055), p=0.064), as well as on day 10–12 (RR=1.012, CI 95% (1.000; 1.023), p=0.098) together with ACE concentration (RR=1.034, CI 95% (1.007; 1.062), p=0.015); $MMWP_{254}$ level (RR=11.195, CI 95% (1.571; 79.771), p=0.016) and $MMWP_{280}$ level (RR=17.370, CI 95% (1.568; 192.455), p=0.02) are significant predictors of a poor outcome on day 1–2 and 5–7 as well ($MMWP_{254}$ — RR=4626.791, CI 95% (7.903; 27808.629), p=0.009 and $MMWP_{280}$ — RR=1331.590, CI 95% (5.006; 354179.342), p=0.012).
CONCLUSION	We identified prognostically significant signs of unfavorable outcomes of septic process: decrease in NOx; growth of ACE concentration; increase in MDA and decrease in TAA; increase in MMWPs; decrease in TAC and EAC.
Keywords:	sepsis, oxidative stress, endogenous intoxication, vascular regulation, predictor of unfavorable outcome.

ACE — angiotensin converting enzyme
ALV — artificial lung ventilation
AOC — antioxidant system
EAC — effective albumin concentration
EI — endogenous intoxication
LP — lipid peroxidation
MDA — malondialdehyde
MMWPs — middle molecular weight peptides
NOx — stable metabolites of nitric oxide
RICU — resuscitation and intensive care unit
RRT — renal replacement therapy
TAA — total antioxidant activity of blood serum
TAC — total albumin concentration

INTRODUCTION

Septic complications remain one of the most important and still unresolved problems of critical care medicine. Infection and associated sepsis cause significant mortality reaching the development of severe sepsis in 55%, and cost multi-billions [1-4]. In 2009, the results of one-day epidemiological study *EPIC II (Extended Prevalence of Infection in Intensive* Care) were published [5], which included data on 13,796 patients from 1,265 resuscitation and intensive care units (RICU) in 75 countries. Infection was detected in 51.4% of patients in the RICU. Risk factors for infection were significant severity of the condition, assessed according to *SAPSII* and *SOFA,* transfers from other hospitals, emergency surgeries, chronic obstructive pulmonary diseases, HIV, immunosuppression, artificial lung ventilation (ALV), renal replacement therapy (RRT). The mortality rate in patients with infection in the RICU was 33.1% and that was higher than in patients without infection (25.3%). This difference was regarded as statistically significant. The hospital stay was longer as well and averaged 29 and 13 days, respectively.

Despite the general criteria developed at the consensus conferences on sepsis in 2002, 2008, 2012 [6-8], and a number of scientific publications devoted to the study of clinical, biochemical and immunological parameters that can help evaluate the risk of death, there are no specific indicators for early diagnosis of septic complications. In 2010, a literature review on the study of sepsis biomarkers was published [9]. The authors studied 3,370 papers, where 178 different biomarkers had been identified, but none of them had sufficient sensitivity and specificity. Therefore, a search of prognostic markers that can be crucial to identify patients with high risk of septic complications and ensure appropriate therapy.

The the aim of this study is to evaluate the prognostic value of biochemical markers of oxidative stress, endogenous intoxication (EI) and vascular regulation in the development of adverse outcomes in patients with sepsis.

MATERIAL AND METHODS

The study included 57 patients with a high risk of septic complications treated at the N.V. Sklifosovsky Research Institute of for Emergency Medicine from October 2010 to November 2012. The study was conducted on day 1-2, day 5-7, and day 10-12 after admission prior to the development of septic complications. In 40 patients, the underlying disease was complicated by sepsis. We examined 30 men and 10 women. The average age of patients was 46.1±12.4 years. In accordance with the criteria of *SSC-2012 (Surviving Sepsis Campaign*), severe sepsis with impaired tissue perfusion and the development of multiple organ failure was diagnosed in 27 patients; 13 patients had signs of septic shock, requiring high doses of pressor amines. The causes of sepsis were generalized fibrinous-purulent peritonitis, acute septic mediastinitis, infectious complications in patients with severe concomitant injury, severe acute pancreatitis in a phase of septic complications. The control group consisted of 17 patients with severe concomitant injury and acute septic mediastinitis, 15 men and 2 women of average age 31.3±12.6 years, where sepsis had not developed. The group with sepsis was significantly older and had greater severity of the condition (Table 1).

Table 1

Comparative characteristics of patients with sepsis and the control group *(M* ±σ)

Parameter	Patients with sepsis *(n*=40)	Control group *(n*=17)	*p*
Age	46.1±12.4	31.3±12.6	0.0001
Gender, m/f	30/10	15/2	0.262
APACHE II, score	19.7±8.0	11.0±3.0	
Source of sepsis			
Generalized fibrinous-septic peritonitis, n (%)	14 (35)	-	
Acute septic mediastinitis, n (%)	10 (25)	1 (5.9)	
Severe concomitant injury, n (%)	8 (20)	16 (94.1)	
ISS, score	49.8±11.6	33.8±6.8	0.004
Severe acute pancreatitis in a phase of septic complications, n (%)	8 (20)	-	
Mortality, n (%)	13 (32.5)	0	

To assess the degree of oxidative stress we used lipid peroxidation (LP) and antioxidant system (AOS) in the blood. The concentration of lipid peroxidation products was assessed according to the level of malondialdehyde (MDA) in the blood serum, which was measured by the method of V.B. Gavrilov [10]. AOC status was evaluated according to the total antioxidant activity of blood serum (TAA), which was measured by the biochemical analyzer (photometric method) *Olympus AU 2700* (*Beckman Coulter*, USA) using reagents of *Randox* (United Kingdom).

Endogenous intoxication (EI) was evaluated by: 1) the level of middle molecular weight peptides ($MMWP_{254}$ and $MMWP_{280}$) in the blood serum, which was measured by the method of N.I. Gabrielyan [11]; 2) total and effective concentration of albumin (TAC, EAC) in the blood serum, which was measured by the fluorescent probe K-35 of the device *ALK-01 ZOND*. [12]

Disorders of endogenous vascular regulation were assessed by the concentration of stable metabolites of nitric oxide (NOx) and angiotensin-converting enzyme (ACE) inhibitors in the blood serum. NOx metabolites were measured reducing nitrate to nitrite by cadmium in the presence of zinc [13]. ACE concentration was evaluated by the biochemical analyzer (photometric method) *Olympus AU 2700* (*Beckman Coulter*, USA) with reagents of *Audit Diagnostics* (Ireland).

Statistical analysis was performed using *Statistica 10.0*. We calculated an average quantity and standard

deviation for normal distribution. The median and interquartile range were calculated upon abnormal deviation. To compare the variables we used the *t-test* of Student for dependent samples or the nonparametric *U*-test of Mann-Whitney. The multivariate assessment of risk factors for death was performed in patients with sepsis using the Cox proportional hazards model. The independent variables were: age, gender, severity of the condition according to *APACHE-II,* indicators of oxidative stress (MDA and TAA), endotoxemia (TAC, EAC, $MMWP_{254}$, $MMWP_{280)}$ and endogenous vascular regulation (NOx, ACE). At the same time, the comparative analysis of these parameters was performed in survived and deceased patients with sepsis and in the control group of patients in order to confirm the effectiveness of determined factors of unfavorable outcomes. The difference was considered as statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

To evaluate the prognostic significance of oxidative stress indicators in the development of adverse outcomes in patients with sepsis, we divided patients into survived/deceased, and conducted a comparative analysis of the data (Table 2). The MDA level both in survived and deceased patients with sepsis was higher on day 1-2 by 1.4 times compared to the control group (statistically significant difference). On day 5-7 and 10-12, the MDA level remained high in all groups. TAA was decreased in deceased patients with respect to its value in the control group on day 1-2, 5-7 and 10-12 (Table 2). Constructing the Cox proportional hazards model we found that the concentration of MDA was not a statistically significant predictor of adverse outcome on day 1-2 (RR=0.745, CI 95% (0.389, 1.4260), p=0.375) and on day 10-12 (RR=0.721, CI 95% (0.379; 1.373), p=0.320) (Table 2). However, the multicenter study by *Lorente L. et al* [14] showed that the risk of death was higher in patients with sepsis, where the MDA level was higher than 4.11 mcmol/l. Our researches also found that the concentration of MDA may be a predictor of adverse outcomes on day 5-7 after admission (RR=1.141, CI 95% (1.033; 1.259), p=0.09) (Table 2). Therefore, according to findings, there was an imbalance in the prooxidant/antioxidant system in patients with severe sepsis and septic shock tending to activation of free radical processes.

The Table 2 shows that on day 10-12, the ACE concentration was higher in the deceased group as compared to survived patients by 1.2 times (statistically significant difference). Constructing the Cox proportional hazards model we found that NOx concentration could be a predictor of poor outcome on day 1-2 (RR=1.026, CI 95% (0.999; 1.055), p=0.064), as well as on day 10-12 (RR=1.012, CI 95% (1.000; 1.023), p=0.098) with ACE concentration (RR=1.034, CI 95% (1.007; 1.062), p=0.015) (Table 2).

Consequently, the regulatory function between the vasodilator NO and vasoconstrictor ACE in critical patients is greatly disturbed within first days of intensive care, which is probably one of the pathogenetic mechanisms leading to changes in microcirculation and eventually to the development of multiple organ failure. Also, the results of our research confirm the development of endothelial dysfunction in sepsis, which manifests as violation of its vasotonic function, resulting in reduced NOx and increased ACE.

The highest value have been placed on studies of endogenous intoxication recently. It is shown that EI develops in all pathological conditions associated with increased catabolism or blocked detoxification systems of the body. The lab parameters of EI may be used in the diagnosis of sepsis and when performing intensive care and selecting its components (extracorporeal management of homeostasis, nutritional support, etc.). The concentration of MMWPs is a biochemical parameter which is most commonly used to assess the EI. MMWPs are endogenous components having a molecular weight of 500-2,000 Dalton (D) formed during proteolysis in damaged tissues, as well as in the plasma when proteolytic enzymes enter the blood. The chemical composition of MMWPs is very heterogeneous and unites a heterogeneous group of substances. For example, activation of free radical oxidation leads to accumulation of toxic substances, which are also regarded as endotoxins. Lipids cleavage products (aldehydes, dialdehydes, epoxides) have a damaging effect on different cell structures, proteins, nucleic acids and other structures. Therefore, these products are endopathogenes. [15].

When comparing survived and deceased patients, there was a statistically significant increase in MMWPs concentration in the deceased group (Table 2). When constructing the regression Cox proportional hazards model we found that the level of $MMWP_{254}$ (RR=11.195, CI 95% (1.571, 79.771), p=0.016), and $MMWP_{280}$ (RR=17.370, CI 95% (1.568; 192.455, p=0.02) is a statistically significant predictor of poor outcomes in critical patients on the first days of the onset of a disease (Table 2), and on day 5-7 ($MMWP_{254}$, RR=4,626.791, CI 95% (7.903; 27,808.629), p=0.009 and $MMWP_{280}$, RR=1,331.590, CI 95% (5.006; 354,179.342), p=0.012).

TAC and EAC are important indicators of oxidative stress. It is known that albumin has detoxifying properties and is involved in sustaining the oncotic pressure of plasma, binding low molecular weight compouns, redox reactions, maintainig ionic equilibrium, regulation of apoptosis, affecting the permeability of endothelium and microcirculation [16]. TAC characterizes only the number of albumin molecules in the sample, while EAC shows a physico-chemical state of albumin globules: the presence of ligands (metabolites, toxins), covalent and non-covalent modification of amino-acid residues, conformation — factors that vary depending on the condition of the body. In our studies, TAC was s reduced by 1.3 times on day 1-2 of intensive care in the group of deceased patients compared to the control group (statistically significant difference) (Table 2). EAC was reduced by 1.3 times in the group of survived patients and by 1.4 times in the group of deceased patients compared to similar data in the control

group (statistically significant differences). On day 5-7, there was no statistically significant difference in TAC among the survived, deceased and patients of the control group, while EAC was lower in the group of deceased patients, compared to values in the control group and the group of survived patients. On day 10-12, TAC and EAC were reduced by 1.4 times in the group of deceased patients compared to the control group (statistically significant difference). When constructing the regression Cox proportional hazards model, we found that EAC could be a predictor of poor outcomes on day 5-7 (RR=0.881, CI 95% (0.768; 1.009), (Table 2). These findings confirm the need for adequate detoxification therapy in critical patients upon admission.

Table 2

Regression Cox proportional hazards model and comparative analysis of indicators in survived and deceased patients

Indicator	Day 1-2					Day 5-7					Day 10-12				
	Relative risk, CI 95 %	*p*	Absolute values			Relative risk, CI 95 %	*p*	Absolute values			Relative risk, CI 95 %	*p*	Absolute values		
			Patients with sepsis		Control group, *n*=17			Patients with sepsis		Control group, *n*=17			Patients with sepsis		Control group, *n*=17
			Survived, *n*=27	Deceased, *n*=13				Survived, *n*=27	Deceased, *n*=13				Survived, *n*=27	Deceased, *n*=13	
Age	1.071 (1.012; 1.113)	0.017	42.7±1.7*, **	53.2±11.1***	31.3±12.6										
APACHE II, score	1.105 (1.023; 1.194)	0.011	16.6±6.5*	24.6±8.1											
TAC, g/l	0.990 (0,935; 1,049)	0.740	31.0 (23.0; 39.0)	27.0 (23.0; 34.0)***	34.0 (31.0; 38.0)	0.951 (0.878; 1.031)	0.224	34.5 (29.0; 38.0)	31.0 (30.0; 32.0)	36.5 (33.0; 41.0)	0.958 (0.885; 1.036)	0.282	37.0 (30.0; 43.0)	32.0 (25.0; 38.0)***	44.0 (41.0; 46.0)
EAC, g/l	0.999 (0.923; 1.082)	0.985	21.0 (17.0; 27.0)**	20.0 (18; 21)***	28,0 (27; 31)	0.881 (0.768; 1.009)	0.068	26.0 (22.0; 32.0)*	22,0 (19.0; 23.0)***	25 (24.0; 28.0)	0.918 (0.810; 1.040)	0.179	26.5 (23.0; 30.0)	22.0 (20.0; 29.0)***	30.0 (29.0; 37.0)
$MMWP_{254}$, relative units	11.195 (1.571; 79.771)	0.016	0.286 (0.245; 0.382)*	0.437 (0.306; 0.608)***	0.288 (0.228; 0.345)	4,626.791 (7.903; 27,808.629)	0.009	0.240 (0.205; 0.260)*	0.299 (0.243; 0.362)	0.259 (0.235; 0.327)	7.292 (0.140; 380.829)	0.328	0.225 (0.186; 0.270)	0.222 (0.214; 0.252)	0,260 (0.227; 0.289)
$MMWP_{280}$, relative units	17.370 (1.568; 192.455)	0.02	0.314 (0.232; 0.146)	0.350 (0.283; 0.677)	0.350 (0.305; 0.480)	1,331.590 (5.006; 354,179,342)	0.012	0.237 (0.222; 0.332)	0.337 (0.254; 0.404)	0,315 (0,212; 0,374)	43.819 (0.243; 7,911.736)	0.154	0.224 (0.182; 0.331)	0.226 (0.193; 0.64)	0.298 (0.198; 0.429)
MDA, mcmol/l	0,745 (0,389;1,4260)	0.375	3.52 (3.04; 4.05) **	3.33 (3.16; 3.77)	2.45 (2.29; 3.03)	1.141 (1.033; 1.259)	0.09	3,45 (3,05;3,88)	3.89 (3.15;4.65)	3.1 (2.80; 3.98)	0.721 (0.379;1.373)	0.320	3.40 (2.72;4.21)	3.45 (2.91; 3.95)	3.42 (3.06; 3.56)
NOx, mcmol/l	1.026 (0.999; 1.055)	0.064	12.5 (8.6; 19.5)	15.5 (7.5; 23.2)	12.9 (12.2; 18.2)	0.841 (0.513; 1.380)	0.494	7,90 (5,69;11,52)	7.41 (5.81;12.57)	7.50 (3.36; 12.98)	1.012 (1.000;1.023)	0.098	6.85 (3.46;19.56)	10.59 (7.53; 11.28)	13.23 (11.24; 16.41)
ACE, mcmol/l	0.993 (0.967; 1.019)	0.587	30.04 (20.85; 45.40)	25.37 (13.82; 36.10)	27.4 (26.4; 36.9)	1.011 (0.982; 1.041)	0.454	30.5 (20.6;37.9)	45.8 (27.5;49.3)***	30.4 (22.1; 36.6)	1.034 (1.007;1.062)	0.015	41.0 (26.6;52.1)*	51.4 (47.3; 76.5)	47.8 (26.5; 48.4)
TAA, mmol/l	1.058 (0.394; 2.840)	0.911	1.72 (1.32; 2.15)	1.38 (1.22; 1.97)	2.17 (1.29; 2.38)	0.686 (0.165; 2.840)	0.603	1.56 (1.22;1.77)	1.16 (0.84;1.87)	1.5 (1.22; 1.97)	0.433 (0.075;2.488)	0.348	1.53 (1.27;1.87)	1.24 (1.02; 1.66)***	2.00 (1.99; 2.21)

Note: * — p<0.05 survived and deceased; ** — p<0.05 survived and control; *** — p<0.05 deceased and control; ACE — angiotensin-converting enzyme; CI — confidence interval; EAC — effective concentration of albumin; MDA — malondialdehyde; MMWP — middle molecular weight peptide; NOx — stable metabolites of nitric oxid; TAA — total antioxidant activity of blood serum; TAC — total concentration of albumin

CONCLUSION

Our results confirm the complexity of the pathophysiology of sepsis and the need for a comprehensive approach in the diagnosis of septic complications. Currently, oxidative stress (particularly, lipid peroxidation) is a universal molecular mechanism of cell damage, aggravated by stress, hypoxia, ischemia and inflammation of various organs and tissues, which may ultimately lead to the development of multiple organ failure [17]. Also, our data support the development of endothelial dysfunction in sepsis, which manifests as violation of vascular tone, resulting in reduced NOx and increased concentration of ACE. In addition, the imbalance between the endogenous factors of cardiovascular regulation (NO and ACE) may cause microcirculation disturbances in patients with sepsis and result in activation of free-radical oxidation. In turn, EI develops under an imbalance between production and elimination of toxic products. Consequently, disturbances of microcirculation may lead to endotoxemia in patients with sepsis.

This is the way how an uncontrolled release of endogenous inflammatory mediators and the lack of mechanisms that limit their damaging effects, cause systemic disorders. For this reason, consideration of sepsis as a systemic response to the infection process reflects the essence of changes [1].

As a result of all the above interactions in the blood of septic patients, the concentration of a huge range of biologically active substances and metabolites alters, that may either play pathological role or be biomarkers for sepsis, or the development and progression of organ dysfunction.

According to blood test findings, prognostically significant signs of poor outcome of sepsis are:

- Reduction of nitric oxide;
- Increasing concentration of ACE;
- Increase in MDA level due to lower total antioxidant activity;
- Increased MMWPs and reduced TAC and EAC.

Changes in these parameters should be necessarily considered when ordering proper therapy.

FINDINGS

1. According to findings, biochemical markers of adverse outcomes in critical patients with sepsis vary depending on the time since the admission.

2. The most important prognostic criteria:

— day 1-2, NOx 15.5 (7.5; 23.2) mcmol/l; $MMWP_{254}$ 0.437 (0.306; 0.608) relative units and $MMWP_{280}$ 0.350 (0.283; 0.677) relative units;

— day 5-7, MDA 3.89 (3.15; 4.65) mcmol/l, $MMWP_{254}$ 0.299 (0.243; 0.362) relative units, $MMWP_{280}$ 0.337 (0.254; 0.404) relative units, EAC 22 (19; 23) g/l.

— day 10-12, NOx 10.59 (7.53; 11.28) mcmol/l, ACE 51.4 (47.3; 76.5) mcmol/l.

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