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ENDOCRINE AND METABOLIC ALTERATIONS MAY UNDERLIE MORTALITY OF SEVERE SEPSIS AND SEPTIC SHOCK PATIENTS ADMITTED TO ICU

By

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Abstract

The study evaluated endocrinal and metabolic response to sepsis and its applicability for the prediction of outcome of septic patients. Patients were 39 adult with severe infections and with- in 24 h after onset of suspected clinical tissue hypoperfusion. At enrollment patients were evaluated for acute physiology and chronic health evaluation II score (APACHE II) and Glasgow Coma Scale (GCS). Global hemodynamic parameters including systolic blood pressure (SBP), heart rate (HR) and central venous pressure (CVP) were recorded and monitored. All patients were managed at ICU due to Surviving Sepsis Campaign guidelines. ELISA estimated serum copeptin, macrophage migration inhibitory factor (MIF) and total cortisol (TC) and blood la ctate levels. Study outcome was survival rate via 28 days (28-D SR) and best predictor for it. The results showed that 22 patients passed total hospital stay uneventfully for a total survival rate of 56.4%. Seventeen patients died; 10 during ICU stay and 7 during word stay. At admission serum markers levels were significantly higher in survivors and non-survivors compared to controls and in non-survivors compared to survivors. Survival showed negative significant cor- relation with age, high blood lactate and serum copeptin, TC and MIF levels. Survival showed positive significant correlation with SBP, CVP and urine output. ROC curve and Regression analyses defined high at admission serum copeptin and blood lactate levels as significant predictors for mortality of septic patients.

Keywords: Septic shock, Total Cortisol, Copeptin, MIF, Survival

Introduction

Sepsis is a systemic response to infection, which may progress to severe sepsis and septic shock (Dellinger *et al,* 2012). Septic shock causes vascular dysregulation making the tissue perfusion dependent on blood pressure. Microvascular perfusion could be disrupted by circulating inflammatory mediators that directly damage peripheral vascular bed (Spanos *et al,* 2010). Microcirculatory dysfunction is documented in the early phase of sepsis and its severity was related to poor outcome (Sakr *et al,* 2004). Arginine vasopressin (AVP), produced by hypothalamic neurons, is stored and released from the posterior pituitary gland following different stimuli especially change in plasma osmolality and stress (Bolignano *et al,* 2014). Reliable measurement of AVP is hindered by several factors. Measurement is subjected to considerable laboratory error because of its short half-life in serum, its instability in withdrawn blood samples and over 90% of AVP is tightly bound to platelets (Jarosz and Maruniak, 2015). Copeptin is a C-terminal part of precursor pre-provasopressin. Activation of the AVP system stimulates copeptin secretion into circulation from the posterior pituitary gland in equimolar amounts with AVP. Copeptin directly reflects AVP concentration and used as a surrogate biomarker of AVP secretion (Struck *et al,* 2005). Increased copeptin concentration was described as a

strong predictor of mortality in patients with chronic and acute heart failure (Łukaszyk and Małyszko, 2015). Severe sepsis activates hypothalamopituitary axis, increasing cortisol production.

Hydrocortisone substitution based on an adrenocorticotropic hormone-stimulation test or baseline cortisol measurement improved sepsis outcome; whether of free cortisol (FC) or total cortisol (TC) is appropriate for critically ill patients is still a matter of debate only due to free fraction of cortisol is active, measurement of FC may be more important than TC in critical patients (Bendel *et al,* 2008). Venkatesh *et al.* (2015) reported that in severe sepsis baseline plasma FC, TC & FC/TC were higher in non-survivors than in survivors and positively correlated with day 90 mortality. Brossaud *et al.* (2015) found FC concentrations obtained by different techniques were significant but not correlated with TC. Molenaar *et al.* (2015) reported that calculated FC levels were too high bias and imprecision for acceptable use in critically ill. Macrophage migration inhibitory factor (MIF) is an important component of the early pro-inflammatory response of innate immune system and is involved in the development of an array of inflammatory disorders including rheumatoid arthritis, inflammatory bowel disease, psoriasis, multiple sclerosis and sepsis (Alam *et al,* 2011). MIF is an immunoregulatory cytokine expressed by variety of different cells and tissues, included immune & non-immune cells. At molecular level MIF interacts with surface CD74 induced phosphorylation and recruitment of CD44; an integral cell membrane glycoprotein with lymphocyte activation role and lymph node homing (Grieb *et al,* 2014).

The current study evaluated the endocrinal and metabolic response to sepsis and its applicability for the prediction of outcome defined as mortality rate.

Patients, Materials and Methods

The current study was conducted at Department of anesthesia & ICU in conjunction with Department of Internal medicine and Department of Clinical and chemical pathology, Aldar Hospital, KSA since Jan. 2013 to Sep. 2015. The study protocol was approved by the Local Ethical Committee and written fully informed patients' consents were signed by the nearest relatives. The study included adult patients with severe infections and within 24 hours after onset of suspected clinical tissue hypoperfusion. Diagnostic criteria for tissue hypo perfusion were 1- systolic blood pressure (SBP) of ≤90 mmHg; 2- urinary output of <0.5ml/kg/min for >2 h; 3- increased heart rate (HR) by ≥10% from baseline; 4- skin presence mottling & 5- hyperlactatemia (>2mmol /L). Patient was considered tissue hypoperfusion on fulfillment of 1 or more of these criteria (Rodriguez *et al,* 2011). Septic shock was severe sepsis induced hypotension persisting despite adequate fluid resuscitation and administration of vasopressors (Levy *et al,* 2003).

At time of enrollment baseline collected data included age, sex and body mass index (BMI) data if possible. Patients were evaluated for acute physiology and chronic health evaluation II score (APACHE II) (Knaus *et al,* 1985) and Glasgow Coma Scale (GCS) (Teasdale *et al,* 1976). Global hemodynamic parameters including SBP, HR & central venous pressure (CVP) were recorded and monitored. Blood gas analysis and pH were obtained simultaneously. All patients were managed at ICU, irrespective of being in need for mechanical ventilation or not, according to Surviving Sepsis Cam-paign guidelines (Dellinger *et al,* 2008). Fluid resuscitation was conducted according to dynamic indices of preload and echocardiography data analysis. Norepinephrine (NE) was used as first line vasopressor in order to achieve a MAP of 65 mm Hg. Positive inotropes (dobutamine or isoproterenol) were used for patients with low cardiac index (Leone *et al,* 2006). Renal replacement therapy was used for patients exhibiting anuria or elevated potassium levels. Room

ambient temperature was at 23-25°C.

Venous blood was taken under aseptic conditions and without use of tourniquet from antecubital vein. Blood sample was clot at room temperature and centrifugation for 20 minutes at 1,000g. Serum was stored at -20°C till ELISA estimation of copeptin (USCN Business Co., USA, Morgenthaler *et al,* 2006). Macrophage migration inhibitory factor (Blue-Gene Biotech, Shan-ghai, China, Potolicchio *et al,* 2003) & total cortisol (ELISA, Cayman Chem. Co, USA, Pradelles *et al,* 1985). Study outcome was survival rate via 28 days (28-D SR) and best predictor for it.

Statistical analysis: Sample power was calculated (Kraemer and Thiemann 1987) by proposed figure to detect a difference at 5% significance level; sample size for 60% power required a N of 30 & for 80% required a N of 41. Sample size and power were recalculated and assured using power and sample size calculation software program provided by Department of Biostatistics, Vanderbilt University. Data were presented as mean with standard deviation, median with IQR at 25 & 75% levels, No. & % and analyzed using One-way ANOVA with post-hoc Tukey HSD test & Chi-square test (X^2 test) for analysis of numbers and ratios. Estimated parameters evaluated as predictors form mortality using receiver operating characteristic (ROC) curve analysis judged by area under curve (AUC) compared versus null hypothesis that AUC=0.05. Regression analysis was used for stratification of parameters as specific predictors. Statistical analysis was done using SPSS for Windows statistical package. $P < 0.05$ was significant.

Results

Of 39 patients fulfilling inclusion criteria; 21 males and 18 females with mean age of 61.2±5; range: 52-69 years. Mean BMI of patients was 26.3±5.1 kg/m^2; 53.8% of patients were overweight-obese, while 28.2% of patients were of average weight and 18% under-weight. Twelve patients had multiple traumas and 27 patients had sepsis either as an indication for surgery or as a postoperative complication (Tab. 1).

Empirical antibiotic therapy started once patient was admitted and after obtaining samples for culture and sensitivity test to define appropriate antibiotic to be given. All patients received fluid therapy with median amount initially was 1800 & 2400 cc at 8 hr thereafter. Twenty-nine patients (74.4%) received norepinephrine at admission time & 18 (46.2%) patients required booster dose of norepinephrine 8 hr thereafter. Nine patients (23.1%) received dobutamine at admission time and five (12.8%) required a booster dose at 8-hr thereafter. Steroid and vasopressin therapy was required for 21 (53.8%) and 6 (15.4%), respectively (Tab. 2)

Table 1: Patients' demographic data

Data			Findings
Age (years)	50-60		11 (28.2%)
	≥60		28 (71.8%)
	Mean (±SD)		61.2±5
Gender	Males		21 (53.8%)
	Females		18 (46.2%)
BMI data	Weight (kg)		75.8±15
	Height (cm)		169.7±3.4
	BMI	Underweight	7 (18%)
		Average weight	11 (28.2%)
		Overweight	14 (35.8%)
		Obese	7 (18%)
		Total	26.3±5.1
Cause of infection	Multiple trauma		12 (30.8%)
	Surgical indication		18 (46.2%)
	Postoperative complication		9 (23%)
Site of infection	Abdominal		19 (48.7%)
	Chest		11 (28.3%)
	Soft tissue infections		9 (23%)

Data as M±SD & numbers; percentages are in parenthesis

Table 2: Therapeutic lines received by patients at time of admission and 8-hr later

		Time	Number (%)	Median
Fluid therapy		T0	39 (100%)	1800 (IQR: 1425-2300)
		T8	39 (100%)	2400 (IQR: 2300-2800)
Vasoactive agent	Norepinephrine (µg/kg/min)	T0	29 (74.4%)	0.18 (IQR: 0.14-0.21)
		T8	18 (46.2%)	0.21 (IQR: 0.17-0.23)
	Dobutamine (µg/kg/min)	T0	9 (23.1%)	8 (IQR: 7-9)
		T8	5 (12.8%)	7 (IQR: 6-9.5)
Steroid (mg/kg/hr)			21 (53.8%)	13.5 (IQR: 12-19)
Vasopressin (U/min)			6 (15.4%)	0.04 (IQR: 0.045-0.058)

Data as numbers and median; percentages and IQR (interquartile ratio 25-75%)

29 patients passed ICU stay favorably and transferred to surgical word, while 10 patients died for anICU mortality (25.6%). 22 patients passed total hospital stay uneventfully and considered as survivors for a total survival rate of 56.4%. Seven died during word stay for amortality (24.1%) of patients admitted alive to surgical word. A total of 17 patients died during hospital stay with mortality rate of 43.4% (Fig. 1).

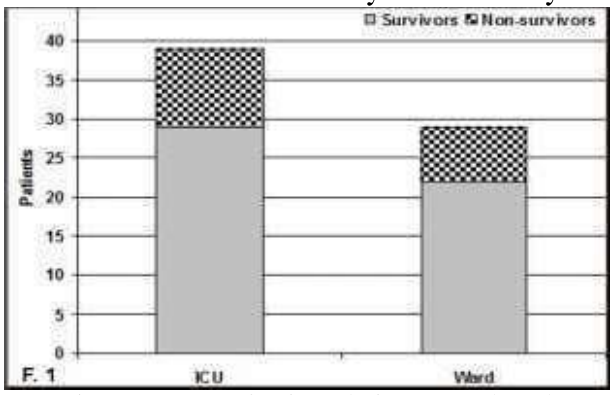

Fig. 1: Outcome of patients during ICU and ward

Table 3: Clinical data of patients categorized according to survival

		Survivors (n=22)	Non-survivors (n=17)	P value
Age (years)		59.4±5.2	63.5±3.8	0.009
Gender; M:F		12:9	19:8	0.206
Temperature (°C)		38±0.5	37.4±0.5	0.011
Pulse rate (beat/min)		101.3±7.9	109.6±11.1	0.029
SBP (mmHg)		91.5±20	86.2±17.9	0.402
UOP (ml/kg/min)		0.4±0.1	0.34±0.06	0.0395
CVP (mmHg)		7.6±0.4	7.3±0.5	0.0486
Oxygenation	SpO_2 (%)	96.5±1.8	95.8±2.2	0.457
data	PaO_2/FiO_2	189.6±35.5	184±22.8	0.051
pH		7.33±0.15	7.29±0.12	0.522
Hemoglobin conc. (%)		9.68±0.65	9.54±0.6	0.875
Blood lactate (mmol/L)		1.8±0.6	2.78±1.22	0.00267
APACHE		24.6±4.2	20.6±1.5	0.001
CGS		13.2±1.7	13.8±1	0.212

P<0.05: significant; SBP: Systolic blood pressure; UOP: Urine output; CVP: Central venous pressure; GCS: Glasgow coma scale

Serum markers levels at time of ICU admission were significantly higher than controls. Estimated serum levels were significantly higher in survivors and non- survivors compared to controls and non- survivors compared to survivors (Tab. 4).

Table 4: Clinical data of studied patients categorized according to survival

		Control	Survivors	Non-survivors
Serum copeptin (pmol/L)	Level	6.9 (4.225-9)	64 (42-81)	145 (83-217)
	P value		P_1=0.001	P_1=0.001, P_2=0.001
Serum total cortisol (pmol/L)	Level	331 (234-394)	672 (471-886)	947 (476-1097)
	P value		P_1=0.0007	P_1=0.00003, P_2=0.0101
Serum MIF (ng/ml)	Level	3.23 (2.01-5.83)	5.91 (4.03-10.77)	7.91 (6.87-12.4)
	P value		P_1=0.047	P_1=0.001, P_2=0.0155

Data as median; intraquartile range between parenthesis; p<0.05: significant difference

Survival showed non-significant correlation with age, high blood lactate & serum copeptin, cortisol and MIF levels. Survival showed positive significant correlation with SBP, CVP & UOP; positive non- significant correlation with APACHE and CGS (Tab. 5).

Table 5: Correlation between survival and clinical and laboratory data of patients

	r	p
Old age	-0.359	0.025
High APACHE	0.289	0.074
High SBP	0.367	0.022
High UOP	0.376	0.018
High CVP	0.318	0.048
High blood Lactate	-0.468	0.003
High GCS	0.224	0.170
High MEDS	-0.384	0.016
High serum Co-peptin	-0.598	0.0007
High serum Cortisol	-0.411	0.009
High serum MIF	-0.376	0.018

SBP: Systolic blood pressure; UOP: Urine output; CVP: Central venous pressure; GCS: Glasgow coma scale; MEDS: mortality in emergency department sepsis; MIF: Macrophage migration inhibitory factor

ROC curve analysis defined high at admission serum copeptin, blood lactate, serum MIF, old age, and high MEDS clinical score, high at admission serum cortisol level, decreased UOP, low SBP and low CVP as significant predicators for mortality in descending order. High at admission serum copeptin and blood lactate levels significantly predictors for mortality among patients (Tab. 6).

Table 6: ROC curve analysis of clinical and laboratory data of patients as predictors for mortality

	AUC	Std. Error	p	CI
Old age	0.721	0.087	0.019	0.549-892
High APACHE	0.350	0.090	0.113	0.174-527
SBP	0.303	0.087	0.037	0.134-0.473
UOP	0.698	0.096	0.036	0.510-0.886
CVP	0.306	0.092	0.040	0.197-0.583
High blood Lactate	0.235	0.081	0.005	0.077-0.393
GCS	0.385	0.093	0.223	0.202-0.568
High MEDS	0.719	0.086	0.020	0.551-0.888
High serum Co-peptin	0.845	0.062	0.0002	0.724-0.966
High serum Cortisol	0.703	0.095	0.031	0.517-0.889
High serum MIF	0.734	0.082	0.013	0.574-0.894

AUC: Area under curve; CI: Confidence interval; SBP: Systolic blood pressure; UOP: Urine output; CVP: Central ve- nous pressure; GCS: Glasgow coma scale; MEDS: mortality in emergency department sepsis; MIF: Macrophage migra- tion inhibitory factor; $p<0.05$: significant.

The regression analysis of significant predictors for mortality was high at admission serum copeptin and blood lactate levels as significant predictors for the mortality of the patients (Tab. 7).

Table 7: Regression analysis of clinical and laboratory data of patients as predictors for mortality

		B	t	p
Model 1	High at admission serum copeptin	-0.554	4.423	0.0006
	High at admission blood lactate	-0.303	2.422	0.021
Model 2	High at admission serum copeptin	-0.598	-4.540	0.0003

Discussion

In the present study, at admission estimated levels of blood markers including blood lactate, and serum copeptin, total cortisol (TC) and MIF were significantly high er in patients compared to controls and in non-survivors than in survivors. In the line with the present results Chuang *et al.* (2014) found that patients with severe sepsis and had fatal outcome within 48-hr exhibit-

ed simultaneously high MIF and IL-10 levels in the early phase of severe sepsis and incremental increases in both IL-10 and MIF levels were associated with rapid fatal outcome in this patient group. Jiang *et al.* (2015) reported significantly lower APACHE II scores and copeptin, C-reactive protein (CRP) and pro- calcitonin concentrations in survivors than in non-survivors. Multiple clinical data and at admission levels of studied blood markers were significantly correlated with mor\tality. But, receiver operating characteristic (ROC) curve analysis defined blood mark- ers as significant predictors for mortality of septic patients than clinical data and scoring. Moreover, Regression analysis for studied blood markers defined high serum copeptin and blood lactate as the most significant predictors for mortality.

These findings indicated the superiority of sepsis-related blood markers over clinical scoring and supported that previously reported using various blood markers, wherein Carpio *et al.* (2015) found high presepsin levels allowed outcome prediction of sepsis patients on admission to a similar degree as mortality in emergency department sepsis (MEDS) and APACHE II scores. Zhao *et al.* (2015) found median complement 3, membrane attack complex (MAC) and mannose-binding lectin levels increased directly with the sepsis, severe sepsis and septic shock groups, and were significantly higher in non-survivors than in survivors and MEDS score and MAC independently predicted in-hospital mortality, but the prognostic performance of MAC was superior to MEDS. Hong *et al.* (2015) found the area under curve (AUC) of the ROC curve analysis of plasma neutrophil gelatinase-associated lipocalin was greater than that of procalcitonin and MEDS score in predicting 28-day hospital mortality.

Unfortunately, none evaluated the studied blood markers including blood lactate, copeptin, cortisol and MIF in combination; however, the obtained results coincided with previous studies evaluated these parameters singularly or in other combinations where Palmiere and Augsburger (2014) reported that copeptin levels were significantly higher in sepsis cases and correlated with procalcitonin, CRP and interleukin-6 (IL-6) values and suggested that hemodynamic instability associated with sepsis and septic shock can be charac- terized by copeptin measurement. Vassili- adi *et al.* (2014) found total cortisol levels relate both to severity and outcome of sepsis and remained fairly unchanged during the illness course and were largely ACTH independent. Tarjányi *et al.* (2014) reported that the predictive values of free cortisol in the first 2 days after admission and total cortisol within 6h are comparable with the complex, routinely used mortality scores in evaluating the prognosis of critically ill patients and concluded that the cortisol response probably reflects the se- verity of disease.

Thomas-Rueddel *et al.* (2015) found that hyperlactatemia increased death risk independent of vasopressor need resulting in different phenotypes within the classic categories of severe sepsis and septic shock. Datta *et al*. (2015) found odd ratios for 30-day death of septic patients' blood lactate level of 2-<4 was double that for patients had blood lactate <2 with survival difference when dividing lactate concentrations into strata and concluded that a single lactate measurement on presentation predicts 30 day mortality independent of other causes. Casserly *et al.* (2015) found that blood lactate level >4 mmol/L was significantly associated with in-hospital mortality than the blood lactate level of 2-3 and 3-4 mmol/L. Dorin *et al.* (2015) re- ported that septic shock was associated not only higher levels but also greater variance of maximal cortisol secretion rate as com- pared to control and sepsis groups. The reported high predictive at admission elevated copeptin and lactate levels for mortality indicated that more precise decision relied using combination of markers not reliance on only one marker. Zhang *et al.* (2014) found that serum copeptin, baseline TC, FC & ACTH concentrations increased gradually with increasing sepsis severity, but multivariate logistic regression analysis showed that copeptin and TC baseline concentrations were independent predictors of septic shock and 28-day mortality and despite of high AUC for copeptin level in septic shock

and 28-day mortality, AUC analysis of combination of copeptin, TC, MEDS score, and procalcitonin level caused significantly prognostic ability than analysis of each parameter. Schuetz *et al.* (2015) found that three biomarkers; proadrenomedullin, copeptin and procalcitonin strongly predicted risk of death, ICU admission and high initial triage priority of septic patients. Jiang *et al.* (2015) found that plasma copeptin, CRP and procalcitonin concentrations positively correlated with APACHE II score in septic patients and reflected disease severity.

Conclusion

Sepsis induced endocrinal and metabolic Alterations manifested as significantly higher serum levels of copeptin & TC and high blood lactate levels. High at admission levels of these markers in conjunction with high serum MIF and clinical scorings could predict 28-day mortality of septic patients, but elevated serum copeptin and blood lactate levels are the most significant predictors.

References

Alam, A, Pal, C, Goyal, M, Kundu, MK, Kumar, R, *et al,* 2011: Synthesis and bioevaluation of human macrophage migration inhibitory factor inhibitor to develop anti-inflamma-tory agent. Bioorg. Med. Chem. 19, 24:7365-73.

Bendel, S, Karlsson, S, Pettilä, V, Loisa, P, Varpula, M, *et al,* 2008: Finnsepsis Study Group: Free cortisol in sepsis and septic shock. Anesth. Analg. 106, 6:1813-9.

Bolignano, D, Cabassi, A, Fiaccadori, E, Ghigo, E, Pasquali, R, ***et al,*** 2014: Copeptin (CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology. Clin. Chem. Lab. Med. 52, 10:1447-56.

Brossaud, J, Gatta, B, Tabarin, A, Corcuff, JB, 2015: Different methods to estimate serum free cortisol: A comparison during cortisol tetracosactide testing. Clin. Chem. Lab. Med. 53, 9: 1367-73.

Carpio, R, Zapata, J, Spanuth, E, Hess, G, 2015: Utility of presepsin (sCD14-ST) as a diagnostic and prognostic marker of sepsis in the emergency department. Clin. Chim. Acta. 450:169-75.

Casserly, B, Phillips, GS, Schorr, C, Dellinger, RP, Townsend, SR, *et al,* 2015: Lactate measurements in sepsis-induced tissue hypoperfusion: results from the Surviving Sepsis Campaign database. Crit. Care Med. 43, 3: 567-73.

Chuang, TY, Chang, HT, Chung, KP, Cheng, HS, Liu, CY, ***et al,*** **2014:** High levels of serum macrophage migration inhibitory factor and interleukin 10 are associated with a rapidly fatal outcome in patients with severe sepsis. Int. J. Infect. Dis. 20:13-7.

Datta, D, Walker, C, Gray, AJ, Graham, C, 2015: Arterial lactate levels in an emergency department are associated with mortality: a prospective observational cohort study. Emerg. Med. J. 32, 9:673-7.

Dellinger, RP, Levy, MM, Carlet, JM, Bion, J, Parker, MM, ***et al,*** **2008:** Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock. Crit. Care Med. 36:296-327.

Dellinger, RP, Levy, MM, Rhodes, A, Annane, D, Gerlach, H, *et al,* 2013: Surviving Sepsis Campaign Guidelines Committee in- cluding the Pediatric Subgroup Surviving Sep- sis Campaign: international guidelines for management of severe sepsis and septic shock. Crit. Care Med. 41:580-637.

Dorin, RI, Qualls, CR, Torpy, DJ, Schrader, RM, Urban FK 3rd, 2015: Reversible increase in maximal cortisol secretion rate in septic shock. Crit. Care Med. 43, 3:549-56.

Grieb, G, Kim, BS, Simons, D, Bernhagen, J, Pallua, N, 2014: MIF and CD74 - suitability as clinical biomarkers. Mini Rev. Med. Chem. 14, 14:1125-31.

Hong, DY, Kim, JW, Paik, JH, Jung, HM, Baek, K, ***et al,*** 2015: Value of plasma neutrophil gelatinase-associated lipocalin in predicting the mortality of patients with sepsis at the emergency department. Clin. Chim. Acta. 452: 177-81.

Jarosz, A, Maruniak, I, 2015: Copeptin-stable C-terminal fragment of preprovasopressin as a new stress marker in newborns. Postepy Hig. Med. Dosw. 69:681-9.

Jiang, L, Feng, B, Gao, D, Zhang, Y, 2015: Plasma concentrations of copeptin, C-reactive protein and procalcitonin are positively correlated with APACHE II scores in patients with sepsis. J. Int. Med. Res. 43, 2:188-95.

Knaus, W, Draper, EA, Wagner, DP, Zimmerman, J, 1985: APACHE II: a severity of disease classification system. Crit. Care Med. 13:818-29.

Kraemer HC, Theimann, S, 1987: How many subjects? Statistical Power Analysis in Research. Newbury Park, CA: Sage.

Leone, M, Boyadjiev, I, Boulos, E, Antonini, F, Visintini, P, *et al,* 2006: A reappraisal of

isoproterenol in goal-directed therapy of septic shock. Shock 26:353-7.

Levy, MM, **Fink, MP, Marshall, JC, Abraham, E, Angus, D, *et al,* 2003:** International Sepsis Definitions Conference. Crit. Care Med. 31, 4:1250-6.

Łukaszyk, E, Małyszko, J, 2015: Copeptin: Pathophysiology and potential clinical impact. Adv. Med. Sci. 60, 2:335-41.

Molenaar, N, Groeneveld, AB, de Jong, MF, 2015: Three calculations of free cortisol versus measured values in the critically ill. Clin. Biochem. 48, 16/17:1053-8.

Morgenthaler, NG, **Struck, J, Alonso, C, Bergmann, A, 2006:** Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin. Chem. 52:112-9.

Palmiere C, Augsburger, M, 2014: Copeptin as a diagnostic biomarker for sepsis-related deaths. Peptides 59:75-8.

Potolicchio, I, Santambrogio, L, Stromінger, JL, 2003: Molecular interaction and enzymatic activity of macrophage migration inhibitory factor with immunorelevant peptides. J. Biol. Chem. 278, 3):30889-95.

Pradelles, P, **Grassi, J, Maclouf, J, 1985:** Enzyme immunoassays of eicosanoids using acetylcholinesterase as label: An alternative to radioimmunoassay. Anal. Chem. 57:1170-3.

Rodriguez A, Lisboa, T, Martín, I, Díaz, E, Trefler, S, ***et al,* 2011:** Mortality and regional oxygen saturation index in septic shock patients: a pilot study. J. Trauma 70, 5:1145-52.

Sakr, Y, Dubois, MJ, De Backer, D, Creteur, J, Vincent, JL, 2004: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit. Care Med. 32:1825-31.

Schuetz, P, Hausfater, P, Amin, D, Amin, A, Haubitz, S, *et al,* 2015: Biomarkers from distinct biological pathways improve early risk stratification in medical emergency patients: the multinational, prospective, observational TRIAGE study. Crit. Care 19:377-82.

Spanos, A, Jhanji, S, Smith, A, Harris, T, Parse, R, 2010: Early microvascular change in sepsis and severe sepsis. Shock 33, 4:387-91.

Struck, J, **Morgenthaler, NG, Bergmann, A, 2005:** Copeptin; a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides 26, 12:2500-4.

Tarjányi, Z, Montskó, G, Kenyeres, P, Márton, Z, Hágendorn, R, ***et al,* 2014:** Free and total cortisol levels are useful prognostic markers in critically ill patients: a prospective observational study. Eur. J. Endocrinol. 171, 6:751-9.

Teasdale, G, **Jennett, B, 1976:** Assessment and prognosis of coma after head injury. Acta Neurochir 34:45-55.

Thomas-Rueddel, DO, Poidinger, B, Weiss, M, Bach, F, Dey, K, *et al,* 2015: Medical Education for Sepsis Source Control and Antibiotics Study Group: Hyperlactatemia is an independent predictor of mortality and denotes distinct subtypes of severe sepsis and septic shock. J. Crit. Care 30, 2:439.e1-6.

Vassiliad, DA, Dimopoulou, I, Tzanela, M, Douka, E, ***et al,* 2014:** Longitudinal assessment of adrenal function in the early and prolonged phases of critical illness in septic patients: relations to cytokine levels & outcome. J. Clin. Endocrinol. Metab. 99, 12: 4471-80.

Venkatesh, B, **Imeson, L, Kruger, P, Cohen, J, Jones, M, *et al,* 2015**: Elevated plasma-free cortisol concentrations and ratios are associated with increased mortality even in the presence of statin therapy in patients with severe sepsis. Crit. Care Med. 43, 3:630-5.

Zhao, X, Chen, YX, Li, CS, 2015: The prognostic performance of the complement system in septic patients in emergency department: a cohort study. Biomark Med. 9, 7:661-8.

Zhang, Q, Dong, G, Zhao, X, Wang, M, Li, C, 2014: Prognostic significance of hypotha- lamic-pituitary-adrenal axis hormones in early sepsis: a study done in emergency department. Intensive Care Med. 40, 10:1499-50