

Brief Report

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*Brief Report*

# Is HSP-27 an Emerging Marker of Good Prognosis in Septic Shock Patients—A Pilot Study

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**Abstract: Objective:** To estimate the value of serum changes of C-reactive protein, procalcitonin, presepsin, heat shock protein 27 (HSP27) and neutrophil to lymphocyte ratio in assessing the prognosis in patients with septic shock (SS) treated in intensive care unit. **Methods:** The SS was diagnosed and treated in accordance with the guidelines of the Surviving Sepsis Campaign. 37 selected adult patients with SS were included. Serum concentrations of biomarkers were measured at admission and daily for 4 consecutive days (time points T0, T1, T2, T3 and T4 respectively). The mortality rate was determined 28 days after admission. Patients were divided into survivor and non-survivor groups according to their mortality. The differences between the levels of biomarkers at the T0 and T4 time points were analyzed. **Results:** The mean value of the SOFA score on admission was  $11.7 \pm 2.7$ , and the APACHE II scale  $29.9 \pm 6.85$ . Nine patients died. Univariate logistic analysis revealed that changes between T0 and T4 time points of presepsin, procalcitonin, and HSP27 were associated with prognosis. A multivariate Cox analysis showed that an increase in HSP27 on T4 was the only independent predictor of good prognosis in SS patients. The area under the receiver operating characteristics curve for HSP27 was 0.785. Kaplan–Meier analysis showed that the mortality was lower ( $p=0.014$ ) in patients who had an increase in HSP27 on T4 compared to those whose serum HSP27 did not increase on T4. **Conclusions:** The increase of HSP27 level on the 4th day predicts favorable outcome in SS patients.

**Keywords:** mortality; sepsis; shock; heat shock protein 27

## Introduction

Sepsis is a life-threatening organ dysfunction caused by an abnormal host response to infection, while septic shock (SS) is a subgroup of sepsis in which abnormalities in circulatory and cellular metabolism are profound enough to significantly increase mortality. Sepsis and septic shock (SS) are one of the main causes of death in critically ill patients. Many biomarkers are used to assess the severity of sepsis and SS, but none are highly sensitive in predicting outcome [1,2]. Moreover, most studies refer to baseline levels of estimated markers rather than changes in markers' levels.

The aim of the study was to estimate the usefulness of changes in some biochemical markers in prognosis of SS.

## Methods

This prospective observational study was conducted in accordance with the Declaration of Helsinki and the study protocol, which was approved by the Institutional Review Board and the Bioethics Committee of Medical University at Lublin, Poland (KE-0254/306/2018). Written informed consent was obtained from all conscious patients or, in case of sedation and mechanical ventilation, from the patients' legal representative.

Included in the study were adult patients with SS. The exclusion criteria were as follows: age < 18 years, more than 24 hours after the onset of symptoms of SS, pregnancy, comorbidities and drugs affecting the immune system, kidney injury requiring dialysis, death during < 48 hours after admission. All patients were treated in accordance with the guidelines of the Surviving Sepsis Campaign [1,2].

Serum levels of biomarkers were measured at admission and daily for 4 consecutive days (time points T0, T1, T2, T3 and T4 respectively). The mortality rate was determined 28 days after admission. Patients were divided into survivor and not-survivor groups according to their mortality. The differences between the levels of biomarkers at the T0 and T4 time points were analyzed. Serum levels of C-reactive protein (CRP), procalcitonin, presepsin, heat shock protein 27 (HSP27) and neutrophil to lymphocyte ratio (NLR) were assessed and analyzed.

The statistical significance of the differences between groups were compared using Student's t-test or using the Mann-Whitney U-test, when appropriate. Cumulative survival curves were constructed by using the Kaplan-Meier method for 28-day mortality. Differences between patient groups were assessed by use of the log-rank test. Independent risk factors affecting prognosis were analyzed by univariate and multivariate logistic regression analysis. Explanatory variables with a p value  $\leq 0.15$  in the univariate analysis were entered into a multivariate analysis. The ROC curve was used to evaluate the diagnostic sensitivity, specificity, and optimal cut-off value. Probability values of  $p < 0.05$  were accepted as significant.

## Results

Out of 48 patients qualified for the study, 11 were excluded due to the need of operation (6), death during < 48 hours after admission (3), lack of all data (2). Remaining 37 patients (24 females and 23 males), aged 57–74 years (mean  $66.1 \pm 7.13$ ) entered the study. 21 patients were treated for SS complicating pneumonia, 10 for SS complicating the postoperative period after gastrointestinal surgery, and 4 for SS complicating urinary tract infection. The mean value of the SOFA score on admission was  $11.7 \pm 2.7$ , and the APACHE II scale  $29.9 \pm 6.85$ . All patients required both ventilation and inotropic agents' treatment. Nine patients (24.3%) died during the follow-up.

Changes in estimated biochemical parameters in subsequent days are presented in Table 1. A decrease of procalcitonin and CRP was observed on day T4 compared to day T0 in both survivors and not-survivors, but the decrease was more pronounced in survivors. For both presepsin and NLR, a decrease was observed only in survivors, while the decrease of NLR was only marginal. In the case of HSP27 an increase on T4 day was observed exclusively in survivors, whereas in non-survivors no changes in HSP27 levels were found. An increase of HSP27 first occurred on T3 day and further sharp increase was observed between T3 and T4 days. Both uni- and multivariate analysis of serum levels changes between T0 and T4 time points for 28-day mortality are depicted in Table 2. Univariate logistic analysis revealed that changes between T0 and T4 time points of presepsin, procalcitonin, and HSP27 were associated with prognosis. A multivariate Cox analysis showed that exclusively an increase in HSP27 in T4 was independent and strong predictor of good prognosis in SS patients. The increase in T4 of 8.92 ng/ml was found the optimal cut-off value of good prognosis. The area under the receiver operating characteristics curve for HSP27 was 0.785 (Figure 1). Kaplan-Meier analysis shown that the mortality was lower ( $p=0.014$ ) in patients who had an increase in HSP27 in T4 compared to those whose serum HSP27 did not increase in T4 (Figure 2).

**Table 1.** Biochemical parameters of the study population grouped into survivors and non survivors. Parameters were estimated for 4 consecutive days (time points T0, T1, T2, T3 and T4 respectively).

Variables	Patients	Time points				
		T0	T1	T2	T3	T4
Procalcitonin (ng/mL)	Total	7.43±3.92	5.94±3.61	4.91±2.68 *	4.19±2.69 **	3.18±2.02 ***
	Survivors	7.19±3.61	5.21±3.26 *	4.44±2.49 **	3.37±2.35 **	2.32±1.52 ***
	Non-survivors	7.76±3.91	6.85±3.65	6.06±2.73	5.86±2.19 *	5.89±2.15 *
Presepsin	Total	1110.6 ±359.2	1120.5±430.3	942.3±330.5	856.7±350.7 *	788.1±310.5**

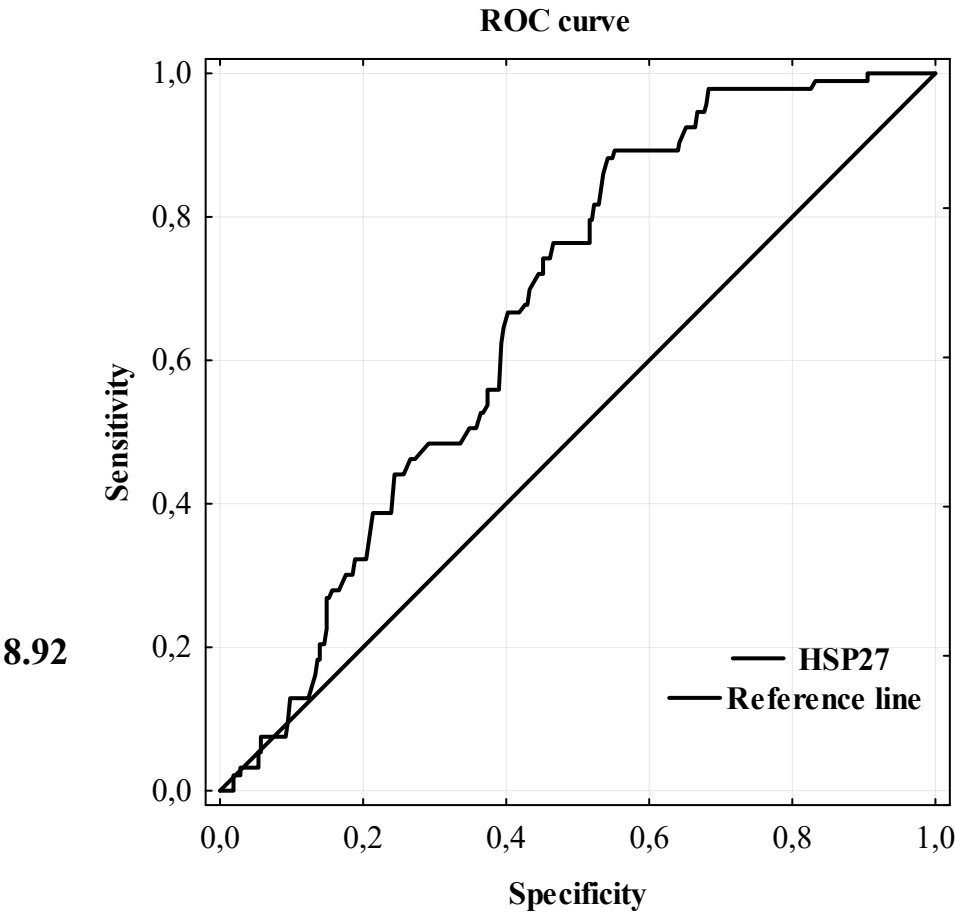
CRP (mg/ml)	Survivors	1100.7±320.8	1108.4±460.1	890.9±270.0 *	805.5±299.4 *	730.4±286.5***
	Non-survivors	1150.3±326.8	1146.6±310.3	938.5±295.2	912.8±370.5	906.8±352.7
	Total	302.3±61	274.3±56	253.6±59 *	207.0±53 **	209.3±39 **
	Survivors	301.2±60	264.5±58	219.6±57 **	201±53 **	200.7±50 **
	Non-survivors	304.8±59	286.1±52	249±56 *	239.1±54 *	231.2±55 *
	Total	5.96±1.75	5.70±1.73	5.65±1.70	7.26±1.76 *	8.29±1.86 **
HSP27 (ng/ml)	Survivors	5.92±1.94	5.78±1.89	5.63±1.89	7.98±1.79 *	10.1±1.82 ***
	Non-survivors	5.55±1.91	5.48±1.87	5.73±1.93	5.69±1.65	5.17±1.81
	Total	19.9±8.22	20.1±8.31	19.3±7.12	17.23±6.03	14.62±7.12 *
NLR (n)	Survivors	19.9±7.97	19.0±8.02	17.86±8.13	16.24±7.56	12.33±7.53 *
	Non-survivors	22.1±8.56	21.9±8.33	21.1±7.99	18.81±6.18	18.15±7.10
	Total					

Values were expressed as means±standard deviation. \* - p value < 0.05; \*\* - p value < 0.010; \*\*\* - p value < 0.001.

**Table 2.** Univariate and multivariate analysis of serum levels changes between T0 and T4 time points for 28-day mortality.

Characteristics	Univariate OR [95% CI]	p-value	Multivariate OR [95% CI]	p-value
Age, years	1.021 [1.014–1.030]	0.003	1.016 [1.010–1.029]	0.009
SOFA (n)	1.309 [1.236–1.334]	<0.001	1.245 [1.211–1.249]	<0.001
APACHE-II (n)	1.175 [1.091–1.213]	0.002	1.037 [1.011–1.043]	0.009
Initial lactate level, mmol/L	1.156 [1.138–1.173]	<0.001	1.139 [1.111–1.166]	0.001
Procalcitonin (ng/mL)	0.955 [0.853–1.208]	0.009	0.961 [0.821–1.965]	0.153
Presepsin (pg/mL)	0.975 [0.898–1.213]	0.013	1.007 [0.715–1.643]	0.206
CRP (mg/ml)	1.651 [1.211–2.931]	0.205		
NLR (n)	1.150 [1.072–1.434]	0.198		
HSP27 (ng/ml)	0.871 [0.805–1.009]	<0.001	0.902 [0.892–1.020]	0.003

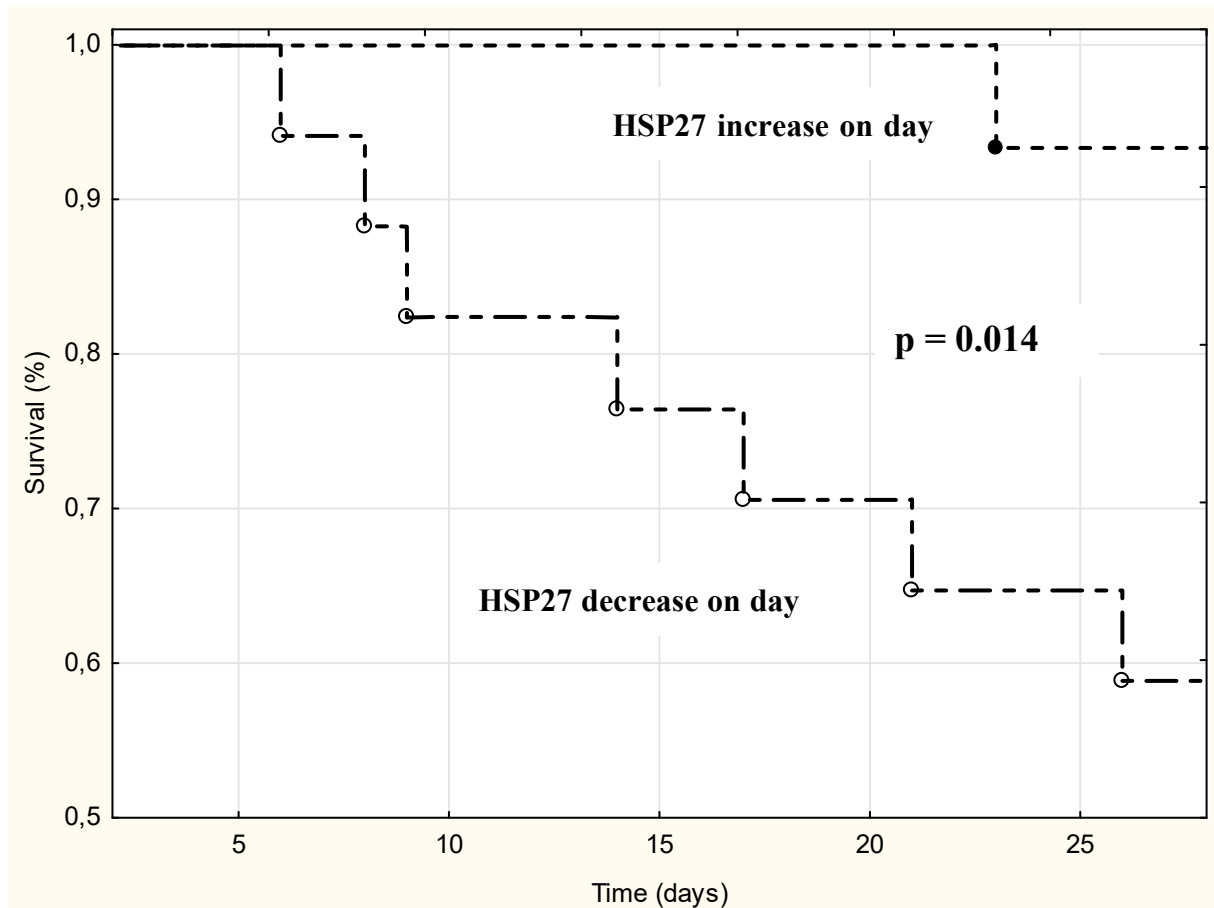
Abbreviations: OR, odds ratio; CI, confidence interval; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation; CRP, C-reactive protein; NLR, neutrophil to lymphocyte ratio; HSP27, heat shock protein 27.



	area	95% CI	sens/spec (%)
HSP27	0.789	0.719-0.823	0.711/0.670

ROC, receiver operating characteristic; HSP27, heat shock protein 27; CI, confidence interval; sens/spec, sensitivity/specificity.

**Figure 1.** ROC curves of HSP27 changes on day 4 of follow-up in predicting mortality in septic shock patients.



**Figure 2.** Kaplan–Meier survival plots for mortality stratified by increase and decrease of serum HSP27 level on day 4.

## Discussion

The main finding of the current study is that an increase in HSP27 level on day 4 is a strong and independent predictor of good prognosis in patients with SS. To our knowledge, this is the first study that has demonstrated the prognostic utility of HSP27 increase for predicting prognosis in patients with SS. HSP27 is associated with cytoprotective functions under conditions of cellular stress and plays important role in the host response to various pathophysiological stresses, such as injury, both oxidative and thermal stress, hypoxia, inflammation, and infections including sepsis. It also plays a role in immune cells activation [3,8]. Data on the role HSP27 plays in sepsis and SS is limited. Recently, it has been demonstrated that in a model of polymicrobial sepsis HSP27 level increase protects mice from sepsis [3]. Moreover, decreased levels of HSP27 were shown in early-onset neonatal sepsis [9]. Enhanced expression of HSPs, including HSP27 was found in activated polymorphonuclear leukocytes in patients with sepsis [10]. The evidence exists that in animal models of endotoxin or septic shock the infusion of glutamine induces the increase of HSP27, attenuates organ injury and improves survival of patients [11–13]. These results suggest potentially protective role of HSP27 against sepsis. Based on our study, we can conclude, however, that an increase in HSP27 is a marker of good prognosis in SS, while determining the potential causal relationship requires further research. The present study has some important limitations. The first limitation is the small number of patients, however, sufficient to show a predictive value for HSP27 levels. The second one is that our study is mostly descriptive, and a pathophysiologic explanation for the results needs to be part of further investigations.

In conclusion, the increase of HSP27 level on the 4th day predicts good outcome in SS patients.

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