Original paper

Is HSP-27 an emerging marker of good prognosis in septic shock patients? A pilot study

Czy HSP-27 jest nowym markerem dobrego rokowania u pacjentów ze wstrząsem septycznym? Badanie pilotażowe

Andrzej Jaroszyński[1], Rafał Wójtowicz[2], Ewa Piasek[3], Wojciech Dąbrowski[3]

[1]*Collegium Medicum*, Jan Kochanowski University, Kielce, Poland
[2]Department of Anesthesiology and Intensive Care, Warsaw South Hospital, Warsaw, Poland
[3]Department of Anesthesiology and Intensive Care, Medical University of Lublin, Lublin, Poland

Medical Studies/Studia Medyczne 2024; 40 (1): 17–21
DOI: https://doi.org/10.5114/ms.2024.137591

Key words: mortality, sepsis, shock, heat shock protein 27, biochemical markers.

Słowa kluczowe: śmiertelność, posocznica, wstrząs, białko szoku cieplnego 27, biochemiczne markery.

Abstract

Introduction: Many biomarkers are used to assess the severity of sepsis and septic shock (SS), but none are highly sensitive in predicting outcome.
Aim of the research: 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 SS treated in an intensive care unit.
Material and methods: Thirty-seven 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 time points T0 and T4 were analyzed.
Results: The mean value of the SOFA score on admission was 11.7 ±2.7, and the APACHE II scale 29.9 ±6.85. Nine patients died. Univariate logistic analysis revealed that changes between T0 and T4 of presepsin, procalcitonin, and HSP27 were associated with prognosis. A multivariate Cox analysis showed that an increase in HSP27 at 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 at T4 compared to those whose serum HSP27 did not increase at T4.
Conclusions: The increase of HSP27 level on the 4th day predicts a favorable outcome in SS patients.

Streszczenie

Wprowadzenie: Do oceny ciężkości przebiegu sepsy oraz wstrząsu septycznego (WS) wykorzystuje się wiele markerów biochemicznych, jednak żaden z nich nie ma wysokiej czułości w przewidywaniu następstw klinicznych.
Cel pracy: Określenie przydatności zmian stężeń w surowicy białka C-reaktywnego, presepsyny, białka szoku cieplnego 27 (HSP27) oraz ilorazu liczby neurocytów i limfocytów w rokowaniu chorych ze WS leczonych na oddziale intensywnej terapii.
Materiał i metody: Do badania zakwalifikowano 37 wyselekcjonowanych dorosłych pacjentów ze WS. Oceny stężenia biochemicznych markerów w surowicy dokonywano w momencie przyjęcia pacjenta na oddział oraz codziennie przez kolejne 4 dni (odpowiednio punkty czasowe T0, T1, T2, T3 i T4). Śmiertelności pacjentów określono po 28 dniach od przyjęcia. Pacjentów podzielono według wskaźnika śmiertelności na grupy osób, które przeżyły oraz zmarły. Analizie poddano różnice stężeń biomarkerów między punktami czasowymi T0 i T4.
Wyniki: Średnia wartość punktacji SOFA w dniu przyjęcia wyniosła 11,7 ±2,7, a skali APACHE II 29,9 ±6,85. W czasie obserwacji zmarło 9 pacjentów. Jednoczynnikowa analiza logistyczna wykazała, że zmiany stężeń między czasem T0 i T4 presepsyny, prokalcytoniny i HSP27 były powiązane z rokowaniem pacjentów ze WS. Wieloczynnikowa analiza Coxa wykazała, że jedynym niezależnym czynnikiem korzystnego rokowania u pacjentów ze WS był wzrost stężenia HSP27 w czasie T4. Pole powierzchni pod krzywą ROC dla HSP27 wynosiło 0,785. Analiza Kaplana-Meiera wykazała, że śmiertelność była mniejsza ($p = 0,014$) u pacjentów, u których wystąpił wzrost stężenia HSP27 w T4, w porównaniu z tymi, u których poziom HSP27 w surowicy nie zwiększył się.
Wnioski: Wzrost stężenia HSP27 w surowicy w czwartym dniu jest korzystnym czynnikiem rokowniczym u pacjentów ze WS.

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 among 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 marker levels.

Aim of the research

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

Material and 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 the 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 ventilated, 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 h after the onset of symptoms of SS, pregnancy, comorbidities and drugs affecting the immune system, kidney injury requiring dialysis, death during < 48 h 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 non-survivor groups according to their mortality. The differences between the levels of biomarkers at the time points T0 and T4 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.

Statistical analysis

The statistical significance of the differences between groups was compared using Student's *t*-test or using the Mann-Whitney *U*-test, when appropriate. Cumulative survival curves were constructed using the Kaplan-Meier method for 28-day mortality. Differences between patient groups were assessed using the log-rank test. Independent risk factors affecting prognosis were analyzed by univariate and multivariate logistic regression analysis. Explanatory variables with a p-value ≤ 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 initially included in the study, 11 were excluded due to the need of operation (6), death during < 48 h after admission (3), or incomplete data (2). The remaining 37 patients (24 females and 23 males), aged 57–74 years (mean: 66.1 ±7.13) entered the study. Twenty-one 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 ±2.7, and the APACHE II scale 29.9 ±6.85. All patients required both ventilation and inotropic agents' treatment. Nine (24.3%) patients died during the follow-up.

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

Discussion

The main finding of the current study is that an increase in HSP27 level on day 4 was a strong and independent predictor of good prognosis in patients

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 [pg/ml]	Total	1110.6 ±359.2	1120.5 ±430.3	942.3 ±330.5	856.7 ±350.7*	788.1 ±310.5**
	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
CRP [mg/ml]	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*
HSP27 [ng/ml]	Total	5.96 ±1.75	5.70 ±1.73	5.65 ±1.70	7.26 ±1.76*	8.29 ±1.86**
	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
NLR (n)	Total	19.9 ±8.22	20.1 ±8.31	19.3 ±7.12	17.23 ±6.03	14.62 ±7.12*
	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

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

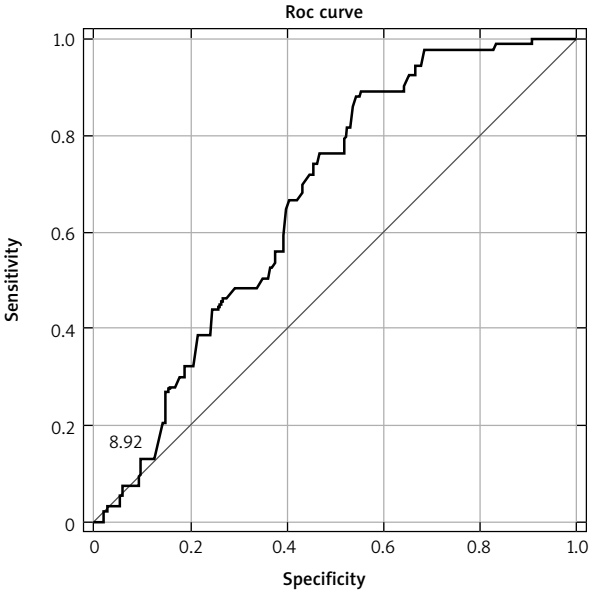
Table 2. Univariate and multivariate analysis of serum level changes between time points T0 and T4 for 28-day mortality

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

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.

with SS. To our knowledge, this is the first study that has demonstrated the prognostic utility of a HSP27 increase for predicting prognosis in patients with SS. HSP27 is associated with cytoprotective functions under conditions of cellular stress and plays an 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 cell activation [3–8]. Data on the role HSP27 plays in sepsis and SS are limited. Recently, it has been demonstrated that in a model of polymicrobial sepsis HSP27 level increase protected mice from sepsis [3]. Moreover, decreased levels of HSP27 were observed in early-onset neonatal sepsis [9]. Enhanced expression of HSPs, including HSP27, was found in activated polymorphonuclear leukocytes in patients with sepsis [10]. There is evidence that in animal models of endotoxin or septic shock the infusion of glutamine induces an increase of HSP27, attenuates organ injury and improves survival of patients [11–13]. These results suggest a 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. If our results are confirmed in future studies, HSP27 may become a marker of prognosis which can be useful in clinical practice. In the future, HSP27 potentially may also become a therapeutic target. The present study has some important limitations. The first limitation is the small number of patients; however, it is 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.



	Area	95% CI	Sens/spec (%)
HSP27	0.789	0.719–0.823	0.711/0.670

HSP 27 Reference line

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

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

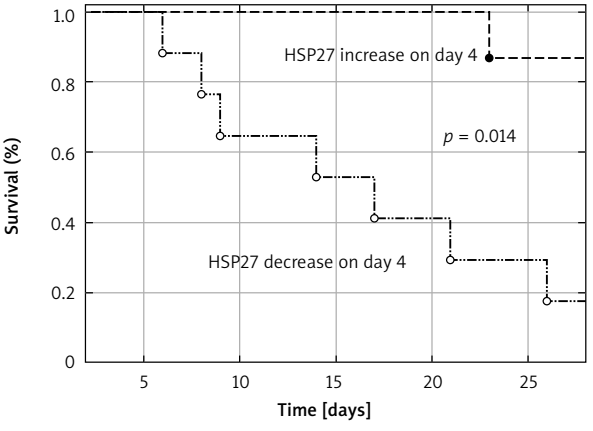


Figure 2. Kaplan-Meier survival plots for mortality stratified by increase and decrease of serum HSP27 level on day 4

Conclusions

The increase of HSP27 level on the 4th day predicts a good outcome in SS patients.

Conflict of interest

The authors declare no conflict of interest.

References

1. Singer M, Deutschman C, Seymour C, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus defnitions for sepsis and septic shock (Sepsis-3). JAMA 2016; 315: 801-810.
2. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith C, French C, Machado FR, Mcintyre L, Ostermann M, Prescott HC, Schorr C, Simpson S, Wiersinga WJ, Alshamsi F, Angus DC, Arabi Y, Azevedo L, Beale R, Beilman G, Belley-Cote E, Burry L, Cecconi M, Centofanti J, Yataco AC, De Waele J, Dellinger RP, Doi K, Du B, Estenssoro E, Ferrer R, Gomersall C, Hodgson C, Møller MH, Iwashyna T, Jacob S, Kleinpell R, Klompas M, Koh Y, Kumar A, Kwizera A, Lobo S, Masur H, McGloughlin S, Mehta S, Mehta Y, Mer M, Nunnally M, Oczkowski S, Osborn T, Papathanassoglou E, Perner A, Puskarich M, Roberts J, Schweickert W, Seckel M, Sevransky J, Sprung CL, Welte T, Zimmerman J, Levy M. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med 2021; 47: 1181-1247.
3. Breed E, Hilliard C, Yoseph B, Mittal R, Liang Z, Chen C, Burd EM, Brewster LP, Hansen LM, Gleason Jr RL, Pandita TK, Ford ML, Hunt CR, Coopersmith CM. The small heat shock protein HSPB1 protects mice from sepsis Sci Rep 2018; 8: 12493.
4. Jaroszyński A, Zaborowski T, Głuszek S, Zapolski T, Sadowski M, Załuska W, Cedro A, Małecka-Massalska T, Dąbrowski W. Heat shock protein 27 is an emerging predictor of contrast-induced acute kidney injury on patients subjected to percutaneous coronary interventions. Cells 2021; 10: 684.
5. Bolhassania A, Agi E. Heat shock proteins in infection. Clin Chim Acta 2019; 498: 90-100.
6. Yu A, Li P, Tang J, Wang J, Chen Y, Liu L. Roles of Hsp70s in stress responses of microorganisms, plants, and animals. Biomed Res Int 2015; 2015: 510319.
7. Zuo D, Subjeck J, Wang X. Unfolding the role of large heat shock proteins: new insights and therapeutic implications. Front Immunol 2016; 7: 75.
8. Vulczak A, Catalão C, de Freitas L, Rocha M. HSP-target of therapeutic agents in sepsis treatment. Int J Mol Sci 2019; 20: 4255.
9. Canul-Euan A, Zúñiga-González G, Palacios-Luna J, Maida-Claros R, Díaz N, Saltigeral-Tigeral P. Increased levels

of plasma extracellular heat-shock proteins 60 and 70 kDa characterized early-onset neonatal sepsis. Front Pediatr 2021; 9: 740274.
10. Hashiguchi N, Ogura H, Tanaka H, Koh T, Nakamori Y, Noborio M, Shiozaki T, Nishino M, Kuwagata Y, Shimazu T, Sugimoto H. Enhanced expression of heat shock proteins in activated polymorphonuclear leukocytes in patients with sepsis. J Trauma 2001; 51: 1104-1109.
11. Wischmeyer E, Kahana M, Ren W, Musch M, Chang E. Glutamine induces heat shock protein and protects against endotoxin shock in the rat. J Appl Physiol 2001; 90: 2403-2410.
12. Singleton K, Serkova N, Beckey V, Wischmeyer P. Glutamine attenuates lung injury and improves survival after sepsis: role of enhanced heat shock protein expression. Crit Care Med 2005; 33: 1206-1213.
13. Zhao Y, Wang H, Liu X, Sun M. Kazuhiro H. Protective effects of glutamine in a rat model of endotoxemia. Mol Med Rep 2012; 6: 739-744.

Address for correspondence:

Andrzej Jaroszyński MD, PhD
Collegium Medicum
Jan Kochanowski University
Al. IX Wieków Kielc 19A
25-317 Kielce, Poland
Phone: +48 604 968 724
E-mail: jaroszynskiaj@interia.pl

Received: 3.08.2023
Accepted: 9.02.2024
Online publication: 25.03.2024