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Is Hyaluran A Biomarker In Patients Without Sepsis-Related Liver Injury?

İlhan Bahar[1*], Gülseren Elay[2], Adnan Bayram[3]

[1]Katip Çelebi University, Atatürk Education and Research Hospital Internal Intensive Care, Izmir, Turkey
[2]Ersin Aslan Training and Research Hospital Intensive Care Anesthesiology, Gaziantep, Turkey
[3]Erciyes University Medical Faculty of Anesthesiology and Reanimation, Kayseri, Turkey

ABSTRACT

The aim of the present study is to evaluate the value of hyaluronan (HA) as a biomarker of sepsis in patients not showing sepsis-associated liver dysfunction and to investigate the relationship between HA level and disease severity.
Sepsis patients that had been followed-up between 2015 and 2016 in intensive care units (ICU) of Erciyes University Hospital were included in this prospective study. Sepsis was defined according to the 2013 Sepsis Survival Campaign Guidelines. The patients were classified as sepsis, severe sepsis, and septic shock. Blood samples were collected from the patients on the first and the third days to measure the HA level. The demographic characteristics, the duration of intensive care unit stay, and mortality data of the patient and the control groups were analyzed.
A total of 46 patients and 30 control subjects were evaluated. HA levels were found to be higher in the sepsis group than the others. HA was neither a predictor of the disease mortality, nor was it correlated with the inflammatory markers, C-reactive protein (CRP), and procalcitonin (PCT).
HA is not a sepsis biomarker in the patients with sepsis who do not show sepsis-associated liver dysfunction.

Key Words: Hyaluronan, sepsis, mortality, liver injury

Introduction

Sepsis affects millions of people around the world every year and kills more than one in four of them (1). Its mortality rates are still high, despite the technical advances (2). One of the most important components of sepsis treatment is early and adequate fluid administration (3). It has been demonstrated that %50 of the cases are fluid-responsive and the other half are not (4). The pathophysiologic mechanism underlying why fluid therapy is not effective in all cases is not clear, excess fluid may result in tissue edema (5,6). Some authors have attempted to explain the intra vascular fluid regulation processes through the glycocalyx (GCX) structures residing on the endothelial surfaces instead of Frank-Starling principles (4). HA, which provides stability to the GCX structures on the endothelial surface, is disrupted as a result of inflammation.

Consequently, the glycocalyx layers heds away (7,8) and the endothelial surface becomes exposed to the shear forces (9), which may then lead to the extravasation of fluid. HA is one of the most important components in terms of the integrity of the endothelial layer through GCX modulation (10) HA acts as a regulator of intra cellular and extra cellular fluid balance (10,11). In a previous study, the large molecular weight HA treatment was used in acute lung injury. HA is predominantly eliminated by the liver (12). It has been suggested as a marker of hepatic fibrosis (13). Since previous studies have not excluded liver failure, it is not clear whether the high HA levels observed have been due to the global organ failure secondary to sepsis, or to liver failure only (14).

We aimed to investigate the effects of global inflammation on HA in sepsis.

Materials and Methods

Study Area: This study was conducted in the various ICUs of Erciyes University Hospital (the general tertiary-level ICU, Anesthesia ICU, Surgical ICU and Internal Medicine ICU). Ethical approval was obtained from the Erciyes University Medical School Ethics Board (2014/388).

Study Design: The patients who had been diagnosed with sepsis between 2015 and 2016 according to the 2012 Sepsis Guideline were included in the study (15). Two blood samples were obtained from each patient, one at the time of sepsis diagnosis hyaluronan1 (HA1)

***Corresponding Author:** İlhan Bahar, Atatürk Eğitim ve Araştırma Hastanesi Basın Sitesi-İzmir, University of Katip Celebi Ataturk Education Hospital, Department of Internal Intensive Care, Izmir, Turkey
E-mail: inb2001@gmail.com, Phone: 0 (232) 243 43 43, Fax: 0 (232) 243 15 30
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Table 1. The laboratory results and mortality rates of the patients

	Sepsis (n= 8)	Severe sepsis (n12)	Septic shock (n=26)	p
Age, Years (mean ± sd)	74.6 ± 4.9	69.9 ± 4.0	63.5 ± 2.7	0.125
Weight, kg (mean ± sd)	81.2 ± 6.7	86.6±5.5	71.3 ± 3.7	0.067
Height, cm (mean ± sd)	160.0 ± 5.9	154.1 ± 4.8	166.3 ± 3.3	0.121
Hyaluronan, median (Q_1-Q_3)	141.6 (67.1-150.1)	50.5 (24.6-117.0)	70.4(24.1-183.0)	<.0001
Admission SOFA score, Median (Q_1-Q_3)	3(1.2- 6)	8(4.2- 9.75)	8.5(6-11)	0.002
Apache II, Median (Q_1-Q_3)	15(11.5-18.5)	24(15-28)	24.5(20-28)	0.003
ICU Stay duration, Days Median (Q_1-Q_3)	6.5(3- 20.5)	12(7- 31)	13.5(8- 22.5)	0.530
ICU Mortality, %, Median (Q_1-Q_3)	50.0%	75%	92.3%	0.023
30-day Mortality, %, Median (Q_1-Q_3)	50.0%	66.7%	92.3%	0.019
90-day Mortality, %, Median (Q_1-Q_3)	62.5%	83.3%	96.2%	0.033

and one on the 3rd day hyaluronan2 (HA2). Being older than 18 years old was the inclusion criteria on for the sepsis patients. The exclusion criteria included the presence of immunosuppression, the presence of liver and/or kidney failure and being in the postoperative period. Patients showing international normalized ratios (INR) above 1.5, activated partial thromboplastin times (aPTT), longer than 60 seconds, or bilirubin levels higher than 4 mg/dl were excluded to exclude liver failure during severe sepsis/septic shock, according to the Sepsis Guidelines (15). A written informed consent was obtained from each patient or a first-degree relative. All patients were followed-up in an ICU environment.

Collection of the blood samples: On the day of sepsis diagnosis according to the Sepsis Guidelines, 3 ml blood samples were collected through an antecubital venous puncture or via arterial line and centrifuged at 1500 Revolutions per minute (rpm) at room temperature. The samples were then transferred into Eppendorf tubes and stored at–80 ˚C, until the day of analysis. As for the control group subjects, following the signature of their written informed consent forms, 3 ml blood samples were taken from antecubital veins, were centrifuged at 1500 rpm at room temperature, and were transferred into Eppendorf tubes to be stored at – 80 ˚C until the day of analysis.

Collection of Data: Data regarding patient age, gender, primary diagnosis, infection site, and physiological scores were recorded. HA levels were measured from each blood sample obtained. Acute Physiologic Assessment and Chronic Health Evaluation (APACHE II) score was calculated using the worst values of the parameters that occurred during first 24 h of ICU admission. Sequential Organ Failure Assessment (SOFA) scores were calculated on the day of the blood sample collection.

Plasma hyaluronat measurement: Plasma HA levels were assessed using a commercial ELISA kit (Cat no: DY3614; R&D Systems). The lowest detectable level of HA by this kit was 1.15 ng/ml. Experiments were performed following the procedures as described by the manufacturer. The fluorometric microplates were read by a spectrophotometer (Gemini XPS, Molecular Devices), and the raw data were analyzed using a dedicated data analysis software (Fluorometric Microplate Spectrophotometer Soft Max PRO).

Statistical Analysis: The data were evaluated using Statistical Package for the Social Sciences (IBM SPSS) Statistics 22.0 (IBM Corp., Armonk, New York, USA) software package. Descriptive statistics were represented as unit numbers (n), percentages (%), means ± standard deviation (x±sd) and median (Q_1-Q_3) values. The normality of the distribution for the numerical variables was assessed with Shapiro-Wilk test and Q-Q charts. Patient and control group comparisons were analyzed using the Student's t- and Mann-Whitney U tests for the variables showing and not showing normal distribution, respectively. One way ANOVA and Kruskal-Wallis tests were used to compare the sepsis groups where the variables showed and did not show normal distributions,

Table 2. The correlation analysis in the patient group

	Sepsis rho(p)	Severe sepsis rho(p)	Septic shock rho(p)
Age	0.48(0.91)	0.77(0.03)	-0.22(0.91)
Weight	0.70(0.05)	0.16(0.60)	-0.02(0.91)
Height	-0.61(0.10)	0.18(0.56)	0.85(0.68)
Apachee II	0.40(0.32)	-0.18(0.55)	-0.10(0.60)
Sofa score	-0.64(0.88)	0.33(0.28)	-0.13(0.95)
Cumulative fluids	0.86(0.84)	-0.21(0.50)	-0.18(0.37)
CRP	-0.17(0.68)	0.33(0.28)	0.11(0.58)
PCT	0.14(0.72)	0.55(0.60)	-0.26(0.19)
Lactate	-0.45(0.26)	0.18(0.57)	0.23(0.91)
Chlorine	-0.27(0.04)	0.16(0.60)	0.16(0.60)
Osmolality	0.96(0.82)	-0.34(0.26)	-0.34(0.26)
Albumin	0.27(0.50)	0.50(0.87)	0.50(0.87)
AST	-0.31(0.45)	0.02(0.93)	0.25(0.93)
ALT	-0.56(0.14)	0.14(0.64)	0.14(0.64)
Bilirubin	-0.62(0.96)	-0.91(0.77)	-0.91(0.77)
Creatinine	0.20(0.62)	0.17(0.58)	0.17(0.58)
Na	-0.45(0.25)	0.40(0.19)	0.40(0.19)
Glucose	0.18(0.67)	-0.22(0.49)	-0.22(0.49)
pH	0.27(0.50)	0.95(0.77)	-0.37(0.12)

Table 3. Demographic Characteristics of the Patient Group and the Control Group

	Patient Group (n=:46)	Control Group (n=30)	p
Age, Years, mean ± SE	68.0(60.0-71.0)	59.0(54.7-62.0)	0.067
Weight, Kg, (Mean ± SE)	70.0(70.0-80.0)	63.0(56.0-71.2)	0.001
Height, cm ± SE	160.0(155.0-170.0)	168.0(163.7-173.2)	0.068
Gender, M, %	25(54.3%)	10(34.4%)	0.10

respectively. For the cases with significant Kruskal-Wallis test results, Dunn-Bonferroni test was recruited to conduct multiple comparisons. The relationships between the numerical variables were evaluated with Spearman correlation test. Kaplan-Meier analysis was used to evaluate the survival rates, and their comparisons were run with Log-Rank test. A Receiver Operating Characteristic (ROC), ROC curve study was performed to establish the optimum HA cut-off values for both the patients and the control subjects. The relationships between the categorical variables were evaluated with chi-square exact test. P values lower than 0.05 were accepted to show statistical significance.

Results

The demographical characteristics of the patients and the control subjects were found to be similar. The SOFA scores at the time of the first blood collection,

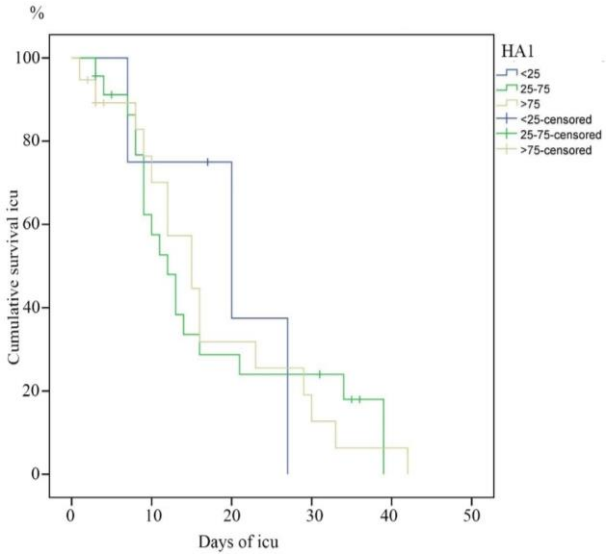

Fig. 1. Hyaluronan %25-75 median (%95 Confidence interval(CI)), 12.0(8.6-15.3), p=0.941)

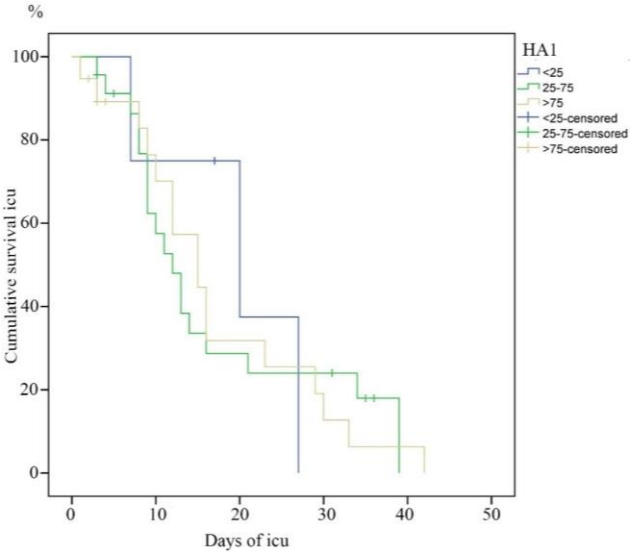

Fig. 2. Hyaluronan %25-75 median (%95 Confidence interval(CI)), 12.0(9.4-14.5), p=0.486)

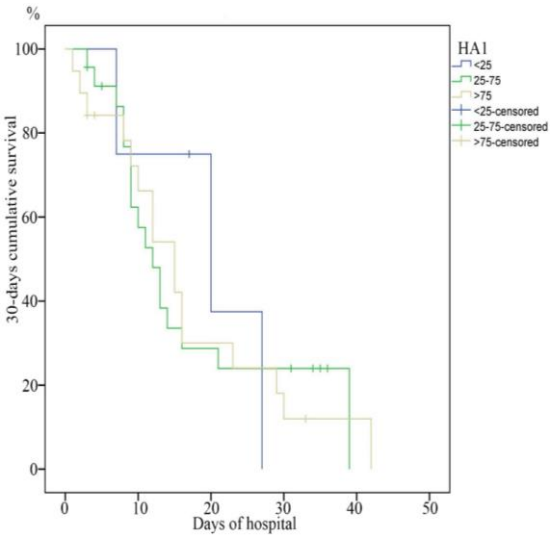

Fig. 3. Hyaluronan %25-75 median (%95 Confidence interval(CI)), 12.0(9.3-14.6), p=0.389)

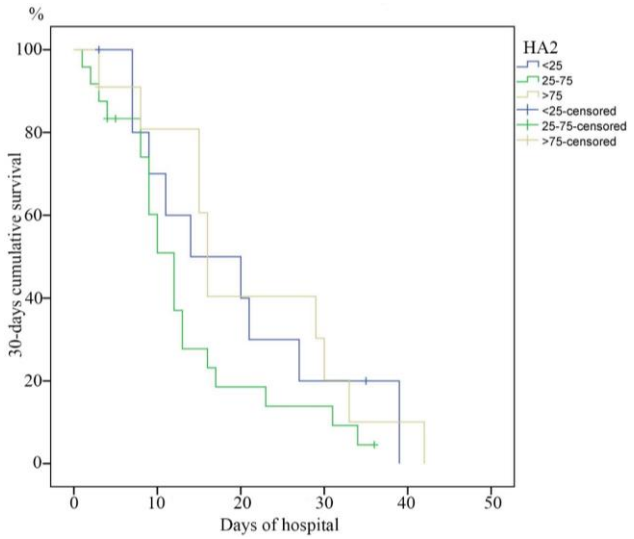

Fig. 4. Hyaluronan %25-75 median (%95 Confidence interval(CI)), 12.0(8.6-15.3), p=0.924)

and the APACHE II scores significantly differed between the patients (p= 0.003 and p= 0.002, respectively). The difference in the body weights of patients and control subjects was statistically significant (p= 0.01) as shown in Table 1.

Regarding correlation analysis among patient groups, the negative correlation with Chlorine was significant (rho, p= -0.27(0.041). When correlation tests were conducted according to various possible factors that can affect HA levels in the patient subgroups, only chlorine levels were found to be negatively correlated with HA in the sepsis patients (p=0.04) as it is in table 2.

The cumulative ICU mortality rates and the 30-day mortality rates were also significantly different between the patient groups. Regarding hyaluronan1, the mean ± SD values of all three patient groups were significantly different when compared to the control group, sepsis and control group (148.42 ± 74.69, 20.63 ± 19.95, p=0.01), severe sepsis and control group (104.13 ± 111.09, 20.63 ± 19.95, p=0.01), septic shock and control group (128.77 ± 112.36, 20.63 ± 19.95, p=0.01). Regarding hyaluronan2, the mean ± SD values of three patient groups were as follows sepsis group (121.27 ±63.37, p=0.01), severe sepsis group (84.24±8.09, p=0.01), septic shock group (102.50 ± 93.34, p=0.01). Among the three patient subgroups, sepsis group showed significantly

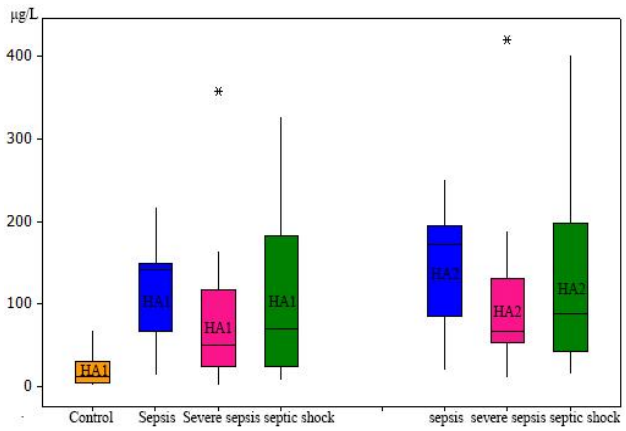

Fig. 5. HA1 and HA2 values of patients with sepsis, severe sepsis and septic shock

higher levels of HA ($p<0.001$). In ROC analysis, the value with 80% specificity and sensitivity was 33.72 ng/dl.

As for the demographical data, the control group subjects had significantly lower body weights than the patients ($p=0.001$), as shown in Table 3.

No significant differences were determined between the three groups regarding the %25-75 and median values of HYA1 and HYA2, ICU and 30-day mortality as shown figure1-2-3-4.

HYA1 and HYA2 levels were significantly higher in the patient group when compared with the control subjects. In the sepsis subgroup, HYA1 levels were statistically significantly higher than the severe sepsis and the septic shock groups ($p=0.01$); however, HYA2 levels were similar between the patients as shown Figure 5.

ROC curve analysis revealed a specificity and sensitivity of 80% at the mean HA level of 33.72 ng/dl is showed Figure 6.

Discussion

In our study, HA was found to be higher in patients with sepsis than in healthy control subjects; however, no significant difference was determined between the patients with sepsis and the patients with severe sepsis or septic shock. Previous studies have reported higher HA levels in the sepsis patients than the patients without sepsis (16,17). These higher levels have been suggested to be attributable to several reasons such as increased HA production and turnover, increased lymphatic outflow and organ failure (16). The relationship between HA levels and severity of sepsis has been evaluated and established previously (14,18). On the other hand, other studies have failed to demonstrate any correlation between disease severity and glycosaminoglycan levels (19,20). Yagmur et al. reported that septic patients with cirrhosis showed

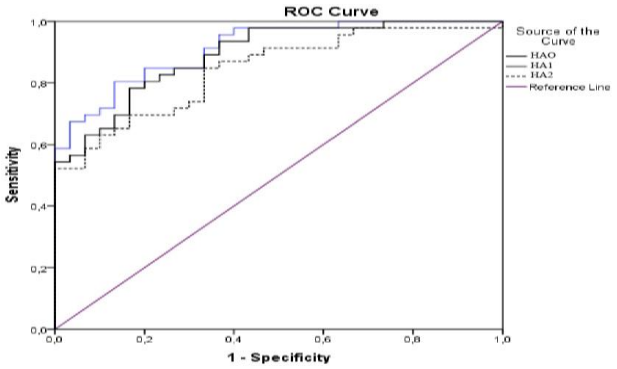

Fig. 6. ROC curve analysis revealed a specificity and sensitivity of 80% at the mean hyaluronan level of 33.72 ng/dl

high levels of HA, and that HA level was not a good predictor of mortality (14). Gudowska M. et al. have reported a close correlation between the HA levels and the presence of liver failure (21). Thus, the high HA levels found by Yagmur et al. might have been due to hepatic dysfunction. Although the patients with liver failure were excluded from this study, the HA levels were still found to be higher in the patients than the control subjects. On the other hand, HA levels were not correlated with the SOFA scores among the patients. In parallel with our findings, Berg et al. also suggested that HA levels might have been affected by the liver functions and that its level might rather be indicative of the liver damage in the patients with sepsis (16). In previous studies, correlations were determined to be present between HA, proinflammatory markers, IL-6, IL-10, and disease severity (22). In the study conducted by Yagmur et al., a correlation was found with PCT and CRP (14). In another study by Köhler et al., correlations were determined between Apache II, SOFA score, and CRP (20). However, we determined no correlation between CRP, PCT, and SOFA score. Also, we were unable to find any relationship of HA with mortality. Jensen et al. suggested that HA might be a marker of sepsis-related liver failure (22). We did not include any patient with liver failure and determined that HA was not a marker in patients with sepsis. The limitation of our study was related to the duration of the study itself; we should have conducted the study in longer durations in the patient groups and should investigate the changes in HA levels during recovery of the patients. Another limitation was the number of the patients; it would be necessary to conduct the study in a larger number of patients.

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References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Garcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29: 1303-1310.
2. Martin GS. Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. Expert Rev Anti Infect Ther 2012; 10: 701-706.
3. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International guidelines for management of sepsis and septic shock: 2016. Intensive Care Med 2017; 43: 304-307.
4. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. Br J Anaesth 2012; 108: 384-394.
5. Marik P, Bellomo R. A rational approach to fluid therapy in sepsis. Br J Anaesth 2016; 116: 339-349.
6. Prowle JR, Kirwan CJ, Bellomo R. Fluid management for the prevention and attenuation of acute kidney injury. Nat Rev Nephrol 2014; 10: 37-47.
7. Kolsen-Petersen JA. The endothelial glycocalyx: the great luminal barrier. Acta Anaesthesiol Scand 2015; 59: 137-139.
8. Schött U, Solomon C, Fries D, Bentzer P. The endothelial glycocalyx and its disruption, protection and regeneration: a narrative review. Scand J Trauma Resusc Emerg Med 2016; 24: 48.
9. Zeng Y, Tarbell JM. The adaptive remodeling of endothelial glycocalyx in response to fluid shear stress. PLoS ONE 2014; 9: e86249.
10. Lennon FE, Singleton PA. Hyaluronan regulation of vascular integrity. Am J Cardiovasc Dis 2011; 1: 200-213.
11. Henry CB, Duling BR. Permeation of the luminal capillary glycocalyx is determined by hyaluronan. Am J Physiol 1999; 277: 508-514.
12. Fraser JR, Engström-Laurent A, Nyberg A, Laurent TC. Removal of hyaluronic acid from the circulation in rheumatoid disease and primary biliary cirrhosis. J Lab Clin Med 1986; 107: 79-85.
13. Adams LA. Biomarkers of liver fibrosis. J Gastroenterol Hepatol 2011; 26: 802-809.
14. Yagmur E, Koch A, Haumann M, Kramann R, Trautwein C, Tacke F. Hyaluronan serum concentrations are elevated in critically ill patients and associated with disease severity. Clin Biochem 2012; 45: 82-87.
15. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013; 39: 165-228.
16. Berg S. Hyaluronan turnover in relation to infection and sepsis. J Intern Med 1997; 242: 73-77.
17. Berg S, Jansson I, Hesselvik FJ, Laurent TC, Lennquist S, Walther S. Hyaluronan: relationship to hemodynamics and survival in porcine injury and sepsis. Crit care Med 1992; 20: 1315-1321.
18. Berg S. Hyaluronan in sepsis. Acta Anaesthesiol Scand 1995; 39: 277.
19. Nelson A, Berkestedt I, Schmidtchen A, Ljunggren L, Bodelsson M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. Shock 2008; 30: 623-627.
20. Köhler M, Kaufmann I, Briegel J, et al. The endothelial glycocalyx degenerates with increasing sepsis severity. Crit Care 2011; 15: 22.
21. Gudowska M, Gruszewska E, Panasiuk A, et al. Hyaluronicacid concentration in liver diseases. Clin Exp Med 2016; 16: 523-528.
22. Jensen JU, Bouadma L. Why biomarkers failed in sepsis. Intensive Care Med 2016; 42: 2049-2051.