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Serum galectin-3 levels predict poor prognosis in sepsis and septic shock patients

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SUMMARY

OBJECTIVE: Sepsis and septic shock are clinical conditions with high mortality and an ever-increasing prevalence, and early diagnosis is of great importance in treating these diseases. Increase in serum Galectin-3 protein in septic patients is associated with increased inflammation, which in turn is associated with mortality. This study aimed to investigate the diagnostic importance of serum Galectin-3 levels and its relationship with in-hospital mortality in sepsis and septic shock patients.

METHODS: This prospective cohort study included 44 sepsis and 44 septic shock patients. Sequential Organ Failure Assessment score and Acute Physiology and Chronic Health Evaluation 2 score were calculated. In addition, routine clinical and laboratory parameters along with serum Galectin-3 were evaluated.

RESULTS: Serum Galectin-3 levels were significantly higher in the septic shock group [4.1 (0.1–10.2) vs. 6.0 (0.1–11.3) ng/mL, respectively; p=0.01]. Moreover, patients with a Galectin-3 level <6.94 ng/mL were associated with longer survival [31.4 vs. 23.1 days; hazards ratio, 1.85; 1.03–3.34, p=0.03]. More importantly, the need for mechanical ventilation, the duration of mechanical ventilation, and serum Galectin-3 levels were independent prognostic factors and predicted poor in-hospital survival in both sepsis and septic shock patients.

CONCLUSION: These findings suggest that Galectin-3 levels are higher in septic shock patients and predict mortality. In addition, high serum Galectin-3 levels, together with mechanical ventilation requirement and mechanical ventilation duration, are closely associated with poor in-hospital survival. Therefore, Galectin-3 may be a valuable diagnostic and prognostic biomarker in these patients.

KEYWORDS: Sepsis. Septic shock. Galectin-3.

INTRODUCTION

Sepsis, defined as an excessive immune response to infection, is a clinical condition with high mortality and an ever-increasing prevalence. Septic shock, on the contrary, is defined as tissue hypoperfusion and fluid-resistant hypotension that require vasopressors and is a more severe clinical condition than sepsis[1-3]. Early diagnosis and treatment are vital for sepsis and septic shock patients. Despite several studies reporting promising results with various biomarkers and scoring systems, it is still unclear which biomarker or scoring system is more functional in daily practice[4-6].

Galectins are beta-galactoside-binding lectins expressed in most living organisms and have critical functions in the immune system. In particular, the Galectin-3 protein is widely expressed in many cells and plays a role in cellular vital functions[7]. It is secreted from damaged and inflammatory cells in diseases, including heart diseases, various infectious diseases, and cancer[8-10]. Moreover, recent studies have reported that it is significantly increased in patients with sepsis and septic shock compared to other biomarkers and is associated with mortality[10,11]. However, the precise role of Galectin-3 in sepsis and septic shock patients has not been fully elucidated yet.

Studies investigating serum Galectin-3 levels in patients with sepsis and septic shock and its relationship with mortality are limited in the literature. Thus, in the present study, we aimed to investigate the importance of serum Galectin-3 levels and its relationship with in-hospital mortality in patients with sepsis and septic shock.

METHODS

Study population

This prospective cohort study enrolled 88 patients diagnosed with sepsis or septic shock in the intensive care unit of The Isparta City Hospital. The study consisted of 44 patients with sepsis and septic shock. Diagnoses of sepsis and septic shock were made according to the guidelines entitled, "the Third International Consensus Definition for Sepsis and Septic Shock

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(Sepsis-3): 2016[3]." The clinical criteria for septic shock were the need for vasopressor therapy to maintain a mean arterial pressure of 65 mm Hg or greater and a serum lactate level greater than 2 mmol/L persisting after fluid resuscitation. Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE) 2 scores were calculated within the first day[3].

We excluded patients with a history of coronary artery disease, congenital heart disease, dysrhythmia, valvular heart disease, heart failure, peripheral arterial disease, hematological disorders, or a history of malignancy. The study was conducted according to the recommendations of the Declaration of Helsinki on biomedical research involving human subjects. It was approved by the Health Research Ethics Board at Süleyman Demirel University.

Blood sample collection

Blood samples were drawn from an antecubital vein by careful venipuncture without stasis before the diagnoses were made on the first day. Hematological indicators were measured within 30 min of collecting the blood samples in tubes containing dipotassium EDTA. Simultaneously, blood gas was taken from the radial artery for diagnosis and treatment. Biochemical analyses were performed with an Olympus AU-640 (Olympus Diagnostica, Hamburg, Germany). An automatic blood counter (Beckman-Coulter Co., Miami, FL, USA) was used for whole blood counts.

Measurement of galectin-3 levels

Serum Galectin-3 levels were measured with the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human Galectin-3. Samples (or standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human Galectin-3 and Avidin-Horseradish Peroxidase conjugate is added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human Galectin-3, biotinylated detection antibody, and Avidin-Horseradish Peroxidase conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a stop solution, and the color turns yellow. The optical density is measured spectrophotometrically at a wavelength of 450±2 nm. The optical density value is proportional to the concentration of human Galectin-3. The concentration of human Galectin-3 was measured by comparing the optical density of the samples to the standard curve.

Statistical analysis

Data were analyzed using the SPSS software version 23.0 for Windows (SPSS, Chicago, IL, USA). Continuous variables were expressed as means±standard deviation or medians and 25th–75th percentile values (normally and non-normally distributed, respectively). To compare continuous variables, the Student's t-test or Mann-Whitney U test was used, as appropriate. Categorical variables were compared using the chi-square test. Using Cox's proportional hazards model, univariate and multivariate analyses for survival differences were performed. Survival was calculated from the diagnosis of the patient to either the date of death from any cause or the date of the last follow-up. Receiver operating characteristic curve analysis was used to determine the cutoff value for serum Galectin-3 levels (6.94 ng/mL). The median cumulative survival probability was calculated using the product-limit method of Kaplan-Meier. Differences in survival between groups were determined using the log-rank test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Baseline demographic and clinical features were comparable between the two groups (Table 1). However, in the septic shock group, while the average heart rate was higher, systolic and diastolic blood pressures were lower. In addition, the need for a mechanical ventilator was significantly higher [24 (54%) vs. 38 (86%), respectively; p<0.01], and mechanical ventilator duration was significantly longer [9.4 (0–55) vs. 16.9 (0–62) day, respectively; p<0.01]. Moreover, the APACHE score (20.4±5.5 vs. 23.6±6.5, respectively; p=0.01) and SOFA score (8.8±2.6 vs. 11.2±3.1, respectively; p<0.01) were remarkably higher (Table 1).

Routine biochemical tests were generally comparable among the groups (Table 1). However, in the septic shock group, the serum C-reactive protein levels [13.4 (3.1–28.5) vs. 17.2 (7.0–28) mg/L, respectively; p<0.01] and lactate level measured from blood gas (2.0±1.1 vs. 3.2±1.9 mmol, respectively; p<0.01 mmol/L) were remarkably higher. Most importantly, serum Galectin-3 levels were significantly higher in the septic shock patients compared with the sepsis patients [4.1 (0.1–10.2) vs. 6.0 (0.1–11.3) ng/mL, respectively; p=0.01].

Survival and prognostic factors

At the last follow-up, the number of patients who died in the septic shock group was higher than in the sepsis group [29 (66%) vs. 20 (45%), p=0.04, Table 1]. In Kaplan-Meier analyses, survival was similar in sepsis and septic shock groups [28.9 vs. 28.2 days; hazards ratio (HR) 0.92; 95% confidence

Table 1. Comparison of demographic, clinical, and laboratory characteristics between the patients with sepsis and septic shock.

	Sepsis n=44	Septic shock n=44	p-value
Mean age, years	79±4	78±6	0.80
Male/female, n/n	26/18	22/22	0.52
Systolic BP, mmHg	112±13	63±4.5	**<0.01**
Diastolic BP, mmHg	65±11	42±3.9	**<0.01**
Heart rate, bpm	100±19	112±24	**<0.01**
Mechanical ventilation, n (%)	24 (54)	38 (86)	**<0.01**
Mechanical ventilation time, days	9.4 (0–55)	16.9 (0–62)	**<0.01**
Hospitalized time, days	17.9 (5–55)	21.8 (5–62)	0.16
SOFA score, n	8.8±2.6	11.2±3.1	**<0.01**
APACHE score, n	20.4±.5.5	23.6±6.5	**0.01**
Mortality, n (%)	20 (45)	29 (66)	**0.04**
Glucose, mg/dL	150±49	147±70	0.82
Creatinine, mg/dL	1.51 (0.3–9.1)	1.38 (0.2–3.4)	0.60
Hemoglobin, g/dL	10.3±2.1	10.0±2.2	0.58
WBC, $\times 10^3$/mL	13.3±6.6	13.6±6.8	0.84
Lymphocyte count, 10^3/mL	1.1 (0.2–4.1)	1.13 (0.1–3.9)	0.86
Neutrophil count, 10^3/mL	11.2 (2.1–27.6)	11.1 (1.5–25.8)	0.84
NLR	15.2 (3.2–89.0)	16.3 (0.3–149.2)	0.78
Procalcitonin, ng/mL	6.0 (0.1–68.2)	7.8 (0.1–94)	0.55
Albumin, g/dL	3.4 (1.6–28.0)	2.6 (1.6–3.8)	0.17
C-Reactive protein, mg/L	13.4 (3.1–28.5)	17.2 (7.0–28.0)	**<0.01**
Galectin-3, ng/mL	4.1 (0.1–10.2)	6.0 (0.1–11.3)	**0.01**
pH	7.40±0.10	7.40±0.09	0.56
PaO_2	69±22	69±18	0.87
$PaCO_2$	43±11	45±10	0.40
HCO_3	27±6	28±7	0.41
Lactate	2.0±1.1	3.2±1.9	**<0.01**

APACHE: acute physiology and chronic health evaluation; BP: blood pressure; HCO_3: bicarbonate; NLR: neutrophil/lymphocytes ratio; pH: acidity/alkalinity; PaO_2: partial pressure of oxygen in arterial blood; $PaCO_2$: partial pressure of carbon dioxide in arterial blood; SOFA: Sequential Organ Failure Assessment Score; WBC: white blood cells. Bold values indicate statistical significance at the p<0.05 level.

interval (CI) 0.51–1.62, p=0.76]. The patients with Galectin-3 level <6.94 ng/mL had prominently longer survival (31.4 vs. 23.1 days; HR 1.85; 95%CI 1.03–3.34, p=0.03, Figure 1).

Additionally, prognostic risk factors were evaluated by univariate analysis (Table 2). According to this analysis, the need for mechanical ventilation (MV, p=0.02), MV duration (p=0.002), neutrophil count (p=0.04), neutrophil-lymphocyte ratio (p=0.002), white blood cells (p=0.02), and serum Galectin-3 level (p=0.005) were significantly associated with survival. Subsequently, all significant prognostic factors were evaluated via multivariate analysis using Cox's proportional hazards model. The need for MV (HR 233; 95%CI 25–2198; p<0.001), MV time (HR 0.86; 95%CI 0.82–0.91; p<0.001), and serum Galectin-3 levels (HR 1.09; 95%Cl 1.01–1.19; p=0.03) were independent prognostic factors and predicted poor in-hospital survival in sepsis and septic shock patients. All multivariate survival analyses are presented in Table 2.

DISCUSSION

In septic shock patients, serum Galectin-3 levels were found to be significantly higher, and levels above 6.94 ng/mL were closely associated with poor in-hospital survival. In addition, the multivariate analysis identified serum Galectin-3 levels,

MV requirement, and MV duration as independent prognostic factors associated with in-hospital survival.

Early diagnosis is of great importance in treating sepsis and septic shock diseases. For this purpose, biomarkers such as procalcitonin, C-reactive protein, cytokine levels (such as IL-6, IL-8, and TNF), and scoring systems such as APACHE 2 and SOFA were studied and shown to be associated with mortality[4-6]. However, despite all these studies, which biomarker or scoring system to prefer in daily practice is still controversial[12]. Mammalian galectins can be found in intracellular and extracellular spaces[7,13]. While the extracellular ones are involved in many extracellular processes such as inflammation and cell-cell communication, the intracellular ones participate in various cellular functions such as anti-apoptosis and cell cycle control. In addition, they take part in critical processes such as cell differentiation, host defense, inflammation, and fibrogenesis[7,13]. Studies in recent years have shown that Galectin-3 is not related to age or body mass index, does not show a circadian rhythm, and increases with exercise but returns to normal after a while[7,13]. A recent study showed that Galectin-3 levels were significantly higher in rats with sepsis due to endotoxemia and played a critical role in the development of systemic inflammation, which is the most important component of the pathophysiology of sepsis[14]. In another study, Ferreira et al. investigated the change in serum Galectin-3 concentrations in mice with sepsis and septic shock induced by cecal ligation and puncture. Similar to our study, they found that serum Galectin-3 levels were significantly higher in the septic shock group than those in the sepsis group. They showed that increased serum Galectin-3 levels in septic rats prevented neutrophil migration to the focus of infection, promoted bacterial spread, and worsened the outcome of sepsis, while Galectin-3 deficiency reduced sepsis-induced organ dysfunction. Moreover, they indicated that these data from rat models are compatible with humans and that high serum Galectin-3 levels are associated with the severity of sepsis, suggesting a new potential biomarker that may be valuable for early diagnosis[15]. Similar to these results, in our study, Galectin-3 levels were significantly higher in septic shock patients than those in sepsis patients and were associated with in-hospital mortality. Likewise, another study reported increased levels of extracellularly released Galectin-3 in the lungs of mice with fatal pulmonary infections with the Francisella novicida strain. They reported that Galectin-3 has immune-modulatory properties such as induction of pro-inflammatory cytokines, immune cell chemotaxis, and regulation of cell death[16,17]. Similar to animal studies, human studies have reported that Galectin-3 is secreted from damaged and inflammatory cells and is an important regulator of the inflammatory response and immune system in heart patients, various infectious patients, and cancer patients, which are associated with poor prognosis. Moreover, it has been suggested as a diagnostic or prognostic marker[7-10,18]. Another study investigated the prognostic value of biomarkers, including presepsin, procalcitonin, and sST2, along with Galectin-3, in sepsis patients. In particular, serum Galectin-3 levels were found to predict 30-day mortality better than the SOFA score and procalcitonin. It has even been argued that the combined use of these markers is more beneficial for the

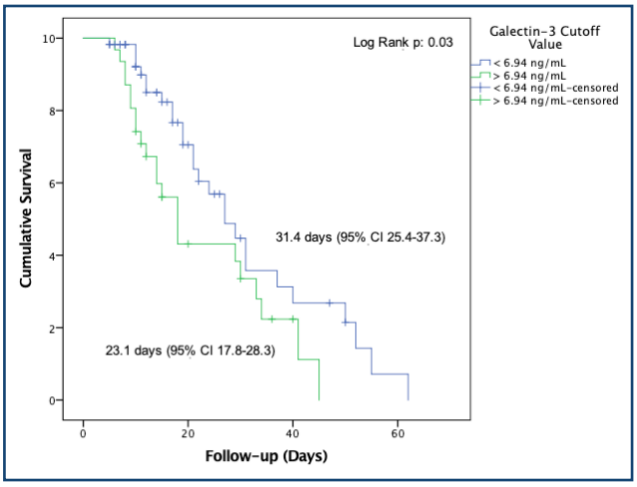

Figure 1. Kaplan-Meier median overall survival curves reflect the difference in survival rates relative to the cutoff galectin-3 values in sepsis and septic shock patients.

Table 2. Results of univariate and multivariate Cox's proportional hazard models regarding overall survival.

Characteristics	Univariate analysis		Multivariate analysis	
	OS HR (95%CI)	p-value	OS HR (95%CI)	p-value
MV	10.4 (1.4–76.1)	0.02	233 (25–2198)	<0.001
MV duration	0.96 (0.94–0.98)	0.002	0.86 (0.82–0.91)	<0.001
Neutrophil count	1.05 (1.00–1.11)	0.04		
NLR	1.02 (1.01–1.03)	0.002		
WBC	1.06 (1.00–1.11)	0.02		
Galectin-3 level	1.09 (1.01–1.18)	0.02	1.09 (1.01–1.19)	0.03

MV: mechanical ventilation; NLR: neutrophil/lymphocyte ratio; OS: overall survival; WBC: white blood cell.

prediction of prognosis[11]. Similarly, in our study, Galectin-3 levels were closely associated with in-hospital mortality in patients with sepsis and septic shock, whereas serum procalcitonin levels, SOFA, and APACHE 2 scores were not. Studies have shown that many sepsis-causing microorganisms contribute to the sepsis process through Galectin-3[19-21].

Several study limitations should be considered. The statistical power of the study may have decreased due to the limited number of patients. Our study does not provide information associated with the long-term results due to the short period of patient follow-up. Only one blood sampling was performed due to the cost. The relationship between serum Galectin-3 and the severity of the disease could be evaluated more clearly by taking multiple blood samples at certain intervals during the course of the disease.

CONCLUSION

Our study showed that serum Galectin-3 levels are higher in septic shock patients than in sepsis patients, and serum levels above 6.94 ng/mL are particularly associated with mortality. Moreover, serum Galectin-3 levels, as well as mechanical ventilator requirement and duration, were closely associated with in-hospital survival. Therefore, we think that Galectin-3 may be a valuable biomarker for early diagnosis and identification of patients with poor prognosis in this disease whose treatment is still not clarified.

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