Research Article / Araştırma Makalesi

# Association of High Mobility Group Box 1 Protein Levels with Sepsis and Outcomes in Newborns

# YeniDoğanlarda High Mobility Group Box 1 Protein Düzeylerinin Sepsis ve Sonuçlarıyla İlişkisi

Tuğba Barsan Kaya, Damla Güneş, Özge Aydemir, Özge Sürmeli Onay, Ayşe Neslihan Tekin

Eskişehir Osmangazi University, Faculty of Medicine, Department of Neonatology, Eskisehir, Türkiye

**Abstract:** High mobility group box-1 protein (HMGB-1) acts as a potent pro-inflammatory cytokine that is actively secreted by innate immune cells and/or passively released by injured or damaged cells during the terminal phase of sepsis. Therefore, serum and tissue levels of HMGB1 are elevated during infection, especially during sepsis. In this study, we aimed to evaluate HMGB1 levels in neonatal sepsis and its association with septic shock and death. Fifty-three neonates with a clinical or proven diagnosis of sepsis were included in the study. Fifty-seven postnatal age-matched neonates without symptoms or signs of infection and receiving routine NICU care were included as controls. Twelve patients had proven sepsis and 6 patients had septic shock. The death occurred in five septic infants. HMGB1 levels were higher in neonates with sepsis compared with controls; patients with septic shock had higher HMGB1 levels compared with those without septic shock (p=0.002). Although non-survivors had higher HMGB1 levels compared to survivors, this was not statistically significant (p=0.086). HMGB1 levels decreased significantly three days after diagnosis in patients without septic shock (p=0.014) but remained elevated in patients with septic shock (p=0.465). A positive correlation was found between CRP and HMGB1 (p=0.008, r=0.252). HMGB1 is a sensitive marker for differentiating patients with sepsis from the non-septic group. The addition of HMGB1 to the group of inflammatory markers may be useful in the detection of patients with severe sepsis compared to the diagnosis of sepsis.
**Keywords:** Biomarker, High mobility group box 1 protein, Newborn, Neonatal Sepsis, Septic Shock

**Özet:** High mobility group box-1 protein (HMGB-1); sepsisin son fazında doğal bağışıklık hücreleri tarafından aktif olarak salgılanan ve/veya yaralı veya hasarlı hücreler tarafından pasif olarak salınan güçlü bir pro-inflamatuar sitokin olarak görev yapar. Bu nedenle, HMGB1'in serum ve doku seviyeleri enfeksiyon sırasında, özellikle sepsis sırasında yükselir. Bu çalışmada, yenidoğan sepsisinde HMGB1 seviyelerini ve septik şok ve ölümle ilişkisinin değerlendirilmesi amaçlandı. Klinik veya kanıtlanmış sepsis tanısı olan 53 yenidoğan çalışmaya dahil edildi. Enfeksiyon semptomu veya bulgusu olmayan ve rutin YYBÜ bakımı alan doğum sonrası yaşı eşleştirilmiş elli yedi yenidoğan kontrol olarak alındı. On iki hastada kanıtlanmış sepsis, 6 hastada septik şok vardı. Beş septik bebek kaybedildi. Sepsisli yenidoğanlarda HMGB1 düzeyleri kontrollere kıyasla daha yüksekti; septik şoklu hastalarda septik şok olmayanlara kıyasla daha yüksek HMGB1 düzeyleri vardı (p=0,002). Hayatta kalmayanların hayatta kalanlara kıyasla daha yüksek HMGB1 seviyelerine sahip olmasına rağmen, bu istatistiksel olarak anlamlı değildi (p=0,086). HMGB1 düzeyleri septik şoku olmayan hastalarda tanıdan üç gün sonra önemli ölçüde düşerken (p=0,014) septik şok gelişen hastalarda yüksek kaldı (p=0,465). CRP ile HMGB1 arasında pozitif bir korelasyon saptandı (p=0,008, r=0,252). HMGB1, sepsisli hastaları septik olmayan gruptan ayırmak için hassas bir belirteçtir. Enflamatuar belirteçler grubuna HMGB1'in eklenmesi, sepsis tanısına kıyasla ciddi sepsisli hastaların tespitinde faydalı olabilir.
**Anahtar Kelimeler:** Biyobelirteç, High mobility group box 1 protein, Yenidoğan, Neonatal Sepsis, Septik Şok

***ORCID ID of the authors:*** TBK. 0000-0003-0698-1850, DG. 0000-0002-8252-2368, ÖA. 0000-0002-4106-0873, ÖSO. 0000-0002-7658-3594, ANT. 0000-0002-2993-5737

***Received*** 13.07.2023 ***Accepted*** 18.09.2023 ***Online published*** 22.11.2023

**Correspondence: Tuğba BARSAN KAYA –** Eskişehir Osmangazi University, Faculty of Medicine, Department of Neonatology, Eskisehir, Türkiye
e-mail : tugbarsan@hotmail.com

## 1. Introduction

Despite advances in perinatal and neonatal care and the use of newer potent antibiotics, sepsis remains a significant cause of morbidity and mortality in neonates. Neonatal sepsis and other severe infections were responsible for an estimated 430,000 neonatal deaths worldwide in 2013, accounting for approximately 15 percent of all neonatal deaths [1]. The prognosis is even worse when sepsis is associated with organ dysfunction.

In sepsis, various pathogen-associated molecular patterns (PAMPs) such as enterotoxins, lipopolysaccharide (LPS), double-stranded RNA, and CpG DNA, as well as damage-associated molecular patterns (DAMPs) such as uric acid, heat shock proteins, annexins, and IL1alpha, can stimulate receptors on immune cells, leading to the release of proinflammatory mediators [2]. Proinflammatory cytokines are responsible for tissue damage, metabolic acidosis, hypotension, multiple organ failure, and death in sepsis.

High Mobility Group Box 1 (HMGB1) is a 30-kDa non-histone nuclear protein that binds to DNA and plays an important role in transcriptional regulation and gene expression [3]. Under inflammatory and injurious conditions, HMGB1 translocates from the nucleus to the circulation and interacts with toll-like receptors (TLR)-2, -4, or the receptor for advanced glycation end-products (RAGE) to function as a potent proinflammatory mediator [3,4]. Due to the slow kinetics of HMGB1 release during sepsis, it is classified as a late-phase cytokine for sepsis [3]. Extracellular HMGB1 acts as an endogenous alarm signal that alerts, activates, and recruits innate immune cells by functioning as a chemokine that facilitates the movement of immune cells to the site of infection [5,6]. Several studies have evaluated the performance of HMGB1 as a biomarker to predict the severity of sepsis in adults [7,8].

This study aimed to evaluate HMGB1 serum levels as a marker for predicting sepsis, septic shock, and their outcomes in neonates.

## 2. Materials and Methods

This prospective study included 53 critically ill term and preterm neonates and 57 controls who were born and admitted to the neonatal intensive care unit of a University Hospital during the period from February 2018 to December 2019. Patients referred to our center and outpatients with suspected sepsis during the study period were enrolled in the study. Infants with matching gestational age (GA) and postnatal age who displayed no symptoms or signs of infection and were receiving routine NICU care were selected as controls. Informed parental consent was obtained for all neonates participating in the study, including the controls. Both patients and controls were enrolled during the same period. Exclusion criteria included perinatal asphyxia, hemodynamically significant heart disease, major congenital anomalies, and surgery within the week prior to sample collection. The Institutional Ethics Committee (IEC) reviewed and approved this follow-up study (IEC study reference number 2017-13 on March 30, 2017). Written informed consent was obtained from parents for each child. The procedures in this study adhered to the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975.

*Definitions*

Sepsis was defined either on the basis of clinical criteria (based on EMA Sepsis scoring)[9] and/or microbiological isolation of organisms on cultures. Patients with positive blood cultures were considered as proven sepsis and those with negative cultures were considered as clinical sepsis. Septic shock was defined as sepsis with cardiovascular dysfunction despite the administration of isotonic intravenous fluid bolus >20-40 mL/kg in 1 h. Cardiovascular dysfunction was described as the presence of hypotension (<5$^{th}$ percentile for age or systolic BP >2 SD below normal for age) or the need for a vasoactive drug to maintain BP in the normal range or at least two of the following: unexplained metabolic acidosis (base deficit >5.0 mEq/L), increased arterial lactate (>2

times upper limit of normal), oliguria (urine output <0.5 mL/kg/h), prolonged capillary refill (>5 s), core to peripheral temperature gap >3°C.[10]

*Measurements*

Blood was collected from a peripheral vein of all neonates at the time of initial laboratory evaluation for sepsis before any treatment. In neonates with sepsis blood was sampled within the first 24 h from sepsis onset and sampling was repeated on day 3. In the control group patients, blood samples for c-reactive protein (CRP) levels, complete blood count and cultures were collected during routine sampling, HMGB1 levels were measured from waste blood samples one time. Serum HMGB-1 levels were measured by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (human HMGB-1 kit Cat. No. E1635Hu, Bioassay Technology Laboratory Shanghai China). The standard curve range was 0.5 ng/ml-150 ng/ml and the sensitivity was 0.24 ng/ml. After centrifugation, serum was kept at -80°C until analysis.

*Clinical data and outcome measures*

Clinical data were collected including prenatal, natal, and postnatal characteristics, presence of septic shock, and death. Blood gases, WBC, CRP, and culture results were also recorded. The primary outcome was a difference in serum HMGB1 concentration between septic and non-septic infants. Secondary outcomes were the association of HMGB1 with CRP and clinical outcomes.

*Statistics*

The statistical data was analyzed using IBM SPSS 21.0 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The normality of variable distribution was assessed by Shapiro–Wilk test. The data were presented as a mean±standard deviation (SD), median (Q1-Q3), frequency and percentage. For the normally distributed groups, the comparisons were performed with t test for two groups. Continuous variables were compared using Mann–Whitney for nonnormal distributed data. Categorical variables were analyzed using the $x^2$ test. All tests of significance were two sided. A p value of ≤0.05 was considered significant The receiver operating characteristic curve (ROC) for HMGB1 values at first values was analyzed to calculate the area under the curve and the most accurate cutoff value for HMGB1.

## 3. Results

Fifty-three patients with sepsis (12 patients with proven sepsis and 41 patients with clinical sepsis) and 57 controls were enrolled in the study. Blood cultures were positive in 10 patients, in 2 patients both of blood and cerebrospinal fluid were positive. Causative organisms were Gram-positive in 8 patients namely *Staphylococcus aureus* (n=5), coagulase-negative *Staphylococcus* spp. (n=3) and Gram-negatives in 4 patients namely *Pseudomonas aeruginosa* (n=1), *Klebsiella* spp (n=2) and *Serratia marcescens* (n=1). The demographic characteristics of the patients are shown in Table-1. Infants with sepsis had significantly lower Apgar scores at the 5$^{th}$ minute (8 [4-10] vs 9 [5-10], p=0.035). There was a higher number of males among infants with sepsis compared to controls (M/F 38/15, p<0.001). the sepsis group had higher rate of respiratory distress syndrome (11 vs 7, p= 0.230) and patent ductus arteriosus (9 vs 2, p=0.02) rates than controls (Table-1).

Serum HMGB1 levels were significantly higher in patients with sepsis compared to controls (35.36 ng/ml vs 22.39 ng/ml p=0.002) (Table 1 and Figure 1). Serum HMGB1 levels were higher in patients with proven sepsis compared to patients with clinical sepsis but it was not statistically significant (p=0.076). Six patients experienced septic shock, and 5 patients died as a result of sepsis. Patients with septic shock had higher HMGB1 levels compared to patients without septic shock (71.21 ng/ml vs 30.78 ng/ml p=0.002) (Figure 2). Despite non-survivors having higher HMGB1 levels compared to survivors, it wasn't statistically significant (65.29 ng/nl vs 32.24 ng/ml p=0.086). Serum HMGB1 levels decreased

significantly 3 days after diagnosis in patients without septic shock (p=.014) however remained high in patients with septic shock (p=0.465) (Table 2). There was a positive correlation between CRP and serum HMGB1 levels (p=0.008, *r*=0.252).

The AUC values are reported with a 95% confidence interval. On the ROC curve analysis HMGB1 performed with an AUC of 0.673 (95% CI 0.57 to 0.77). The best discriminative cut-off value of HMGB1 was 12.42 ng/ml for sepsis (sensitivity 81%, specificity 53%) (Figure-2). CRP had better sensitivity and specificity for the diagnosis of sepsis than HMGB1 in ROC analysis (0.971 (0.935-1.000) 95%CI) (Figure-3)

**Table 1.** Clinical characteristics of study groups

| **Parameter** | **Infants with sepsis (n= 53)** | **controls (n= 57 )** | **p** |
|---|---|---|---|
| Birth weight (gram)* | 2362± 1073 | 2086±703 | **0.013** |
| Gestation age (week)** | 34 (24-40) | 35 (24-40) | 0.083 |
| Gender (M/F) | 38/15 | 22/35 | **<0.001** |
| Cesarean section | 31 | 45 | **0.039** |
| 5. min. Apgar score** | 8 (4-10) | 9 (5-10) | **0.035** |
| Antenatal steroids (n) | 19 | 15 | 0.370 |
| Postnatal age (day)** | 7.5 (3-20) | 7 (5-11) | 0.445 |
| Preterm premature rupture of membranes (n) | 7 | 7 | 0.571 |
| Respiratory Distress Syndrome (n) | 11 | 7 | 0.230 |
| Patent ductus arteriosus (n) | 9 | 2 | **0.025** |
| Bronchopulmonary Dysplasia (n) | 5 | 2 | 0.259 |
| Necrotising Enterocolitis (n) | 3 | 1 | 0.350 |
| Intraventricular hemorrhage (n) | 2 | 0 | 0.230 |
| Retinopathy of prematurity (n) | 4 | 3 | 0.707 |
| HMGB1 (ng/ml)* | 35.36±30.0 | 22.39±21.1 | **0.002** |
| Mortality (n) | 5 | 0 | **0.018** |

**Table 2.** First and 3$^{th}$ day serum HMGB1 levels in infants with septic shock and without septic shock

| **Test Result Variable** | **1$^{st}$ day** | **3$^{rd}$ day** | **p** |
|---|---|---|---|
| HMGB1 in sepsis with shock (ng/ml)* | 71.64 (44.10-91.79) | 81.63 (34.22-96.41) | 0.465 |
| HMGB1 in sepsis without shock (ng/ml)* | 15.35 (6.16-102.63) | 12.60 (7.8-84.74) | **0.014** |

*Median (minimum maksimum)

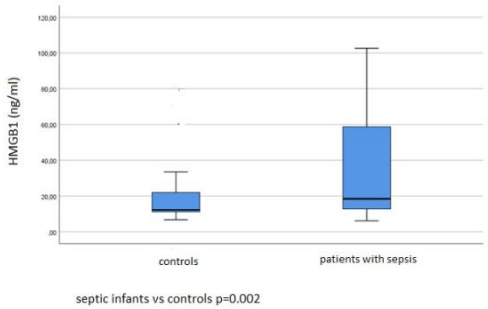


***Figure 1.*** *Box-plot of high-mobility group box-1 (HMGB1) levels in healthy controls and patients with sepsis. Central horizontal line is the median, box borders represent the interquartile range and whiskers represent the 5th and 95th percentiles.*

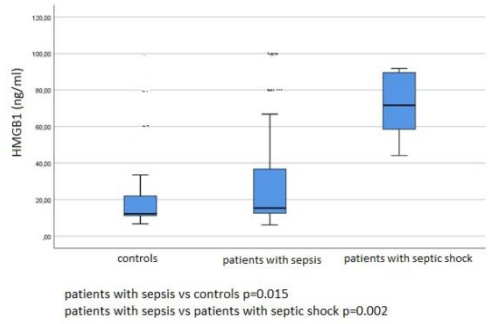


***Figure 2.*** *Box-plot of high-mobility group box-1 (HMGB1) levels in healthy controls, patients with sepsis, and septic shock. Central horizontal line is the median, box borders represent the interquartile range and whiskers represent the 5th and 95th percentiles.*

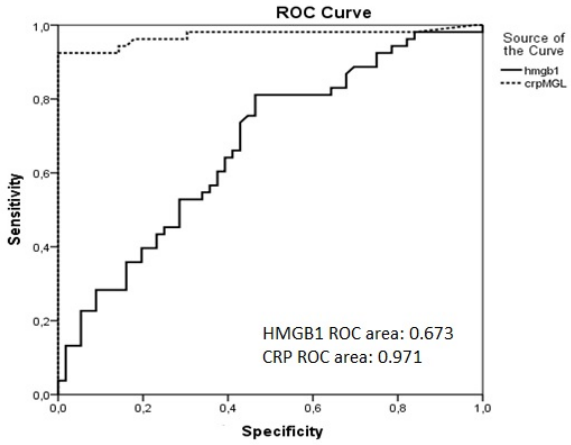


***Figure 3.*** *ROC curve analysis for HMGB1 and CRP between infants with sepsis and controls (AUC: Area under the curve)*

## 4. Discussion

In this prospective study on neonates, our aim was to demonstrate the diagnostic performance of HMGB1 in sepsis and its association with disease severity. HMGB1, originally named amphoterin is considered an important delay-phase cytokine in sepsis.[3,12] Wang et al.[3] showed the relationship of HMGB1 with septic shock in mice models. They reported a significant increase in HMGB1 levels in cases of severe sepsis. Administration of exogenous HMGB1 to septic mice increased mortality, and administration of antibodies against HMGB1 ameliorated the clinical outcome of septic animals.[3] Following this report, HMGB1 levels have been measured in several clinical cohorts to evaluate whether this protein is involved in human sepsis. The common thread in all these studies is that the patients with sepsis have higher HMGB1 levels compared to the controls.[8,12-16] To our knowledge, this study is the first to investigate HMGB1 levels in neonatal sepsis. In agreement with Wang et al.[3] we found that HMGB1 levels are significantly higher in infants with sepsis compared to controls. Although this result confirms a proinflammatory role of HMGB1 in sepsis, HMGB1 did not perform well in a ROC analysis examining its ability to identify septic infants, achieving an AUC of only 0.67 on day 1. The ability to identify patients with sepsis, CRP performed better with an AUC of 0.97. Non-survivors had higher HMGB1 levels than survivors, although the difference was not statistically significant. This could be explained by the small number of patients who died. The most striking result of our study was that while the HMGB1 level decreased in patients without septic

shock on day 3, it remained high in patients with septic shock. These results were like those obtained by Gibot et al.[17] at investigate plasma HMGB1 concentration during septic shock. In this study HMGB1 concentration tended to display peak on day-3 in septic shock non-survivors, while survivors showed decreased after day-1. In another study by Karakike et al.[18] was assessed the impact of HMGB1 kinetics on mortality in adult patients. Serial measurements revealed that delayed and persistent HMGB1 release were associated with a worse prognosis, especially in patients with underlying chronic inflammatory conditions. Angus et al.[13] confirmed that HMGB1 levels are frequently elevated in patients with severe sepsis and is higher in non-survivors. They observed an increase in HMGB1 levels over time in non-survivors. Early diagnosis of systemic infection and sepsis can be challenging due to the variability and inconsistency of clinical indicators, which lack uniformity and specificity. In some cases, sepsis can progress to severe sepsis or septic shock. Better diagnostic, prognostic, and immunological molecular markers are needed for the detection of infection and the degree of inflammation. Based on our findings, we hypothesize that serial HMGB1 measurements in neonatal sepsis may serve as a prognostic indicator rather than a diagnostic marker.

In our study, the rate of patients with preterm rupture of membranes was equal in both groups. Nevertheless, because we did not assess amniotic fluid for infection or inflammation in our study, we cannot make any comments regarding the potential impact of preterm rupture of membranes on postnatal HMGB1 levels.

HMGB1 levels can be measured by ELISA or blotting methods. HMGB1 levels assessed by ELISA are much lower compared to those measured by blotting methods.[3,7,13] In the present study, we used ELISA to determine the serum concentration of HMGB1 and observed higher levels compared to adult studies. Gaini et al.[14] reported median HMGB1 levels of 4.3 ng/ml in the sepsis group, 6.7 ng/ml in the severe sepsis group, and 4.8 ng/ml in septic shock. A study by Yasuda et al.[19] evaluating the contribution of serum HMGB1 levels in patients with severe acute pancreatitis observed mean HMGB1 levels in patients of 5.4±1.3 ng/ml. In another study, the median HMGB1 level is in Burkholderia pseudomallei sepsis was 11.1 ng/ml.[20] Only few studies in the literature have investigated HMGB1 levels in neonate patients. HMGB1 levels were measured by the ELISA method in both studies. In the study by Tang et al.[21] patients with neonatal hypoxia-induced persistent pulmonary hypertension had HMGB1 levels of 33.19±9.45 ng/ml. The other study on neonates has evaluated the relationship between the intensity of fetal inflammation and HMGB1 levels at birth.[22] Median HMGB1 levels in the amniotic fluid were 21.4, 15.9, and 21.2 ng/ml in absent, mild and severe inflammation respectively. The Nakamura et al.[23] study established a reference range for neonatal HMGB1 levels (2.0-35.3 ng/ml).

HMGB1 levels obtained in these studies were much higher values than adult studies as in the present study. The profile of pro- and anti-inflammatory cytokines, in neonate infants is complex and not fully understood. Elevated HMGB1 levels in neonates could be associated with stress-induced inflammation due to birth and/or an overproduction of proinflammatory cytokines in response to infection.[22-26]

Little is known about the cytokine levels in non-infected neonate infants both preterm and term neonates. In a study investigating immunological biomarker concentrations in cord blood, 12 biomarkers (IL-2, IL-4, IL-5, IL-8, IL-10, MCP-1, MIP-1a, MIP-1b, sIL-6ra, sTNF-RI, TNF, and TREM-1) were shown to be higher in preterm neonates compared to term, but IL-1b and IL-18 were lower.[27] The main limitation of the present study is the lack of data on HMGB1 levels in the first weeks of life in neonates and the impact of gestational age on HMGB1. In addition, the study group was not homogeneous due to the enrollment of both term and preterm infants and also early and late neonatal sepsis cases. More meaningful results can be achieved with a specific group of sepsis patients, and interpretation of the

results should consider the progression of HMGB1 levels in the first weeks of life. More research using sequential measurements such as those in the early weeks of life and in neonates of varying gestational ages, is required to determine HMGB1 levels.

In summary, we have demonstrated elevated HMGB1 levels in neonatal sepsis. Furthermore, HMGB1 levels were higher in infants with more severe diseases presenting as septic shock. Increased serum concentration provides evidence of a proinflammatory role for HMGB1 in sepsis. Additional research with a more homogeneous study group and a larger sample size is needed to better understand the role of HMGB1 in patients with sepsis and organ dysfunction.

## REFERENCES


1. Oza S, Lawn JE, Hogan DR, et al. Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000-2013. Bull World Health Organ. 2015; 93:19.
2. Wiersinga WJ, Leopold SJ, Cranendonk DR, et al. Host innate immune responses to sepsis. Virulence. 2014;5(1):36-44.
3. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248-251.
4. Yang H, Wang H, Czura CJ, et al. The cytokine activity of HMGB1. J Leukoc Biol 2005;78:1-8
5. Yang H, Wang H, Tracey KJ. HMG-1 rediscovered as a cytokine. Shock. 2001;15:247-253.
6. Andersson U, Wang H, Palmblad K, et al. High Mobility Group 1 Protein (HMG-1) Stimulates Proinflammatory Cytokine Synthesis in Human Monocytes. J Exp Med. 2000;192:565-570.
7. Sundén-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Critical care medicine. 2005;33(3):564-573.
8. Karlsson S, Pettila V, Tenhunen J, et al. HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis. Intensive Care Med. 2008;4:1046-1053.
9. European Medicines Agency (EMA). Report on the Expert Meeting on Neonatal and Paediatric Sepsis London: 2010. [updated 8 June 2010]. Available at: www.ema.europa.eu/en/documents/report/report-expert-meeting-neonatal-paediatricsepsis_en.pdf
10. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005;6:2-8.
11. Parkkinen J, Raulo E, Merenmies J, et al. Amphoterin, the 30- kDa protein in a family of HMG1-type polypeptides. Enhanced expression in transformed cells, leading edge localization, and interactions with plasminogen activation. J Biol Chem. 1993;268:19726-19738
12. Huang LF, Yao YM, Dong N, et al. Association of highmobility group box-1 protein levels with sepsis and outcome of severelyburned patients. Cytokine. 2011;53:29-34.
13. Angus DC, Yang L, Kong L, et al. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. Critical care medicine. 2007;35(4):1061-1067.
14. Gaini S, Pedersen S, Koldkjaer O, et al. High mobility group box-1 protein in patients with suspected community-acquired infections and sepsis: a prospective study. Crit Care. 2007;11(2):1-10.
15. Xin K, Sun J, Liu P, et al. Expression and significance of HMGB1 in patients with sepsis and effects on prognosis. All Life. 2020;13(1):164-170.
16. Gaini S, Koldkjaer OG, Moller HJ,et al. A comparison of highmobility group-box 1 protein, lipopolysaccharide-binding protein and procalcitonin in severe community-acquired infections and bacteraemia: a prospective study. Crit Care. 2007;11(4)1-10.
17. Gibot S, Massin F, Cravoisy A, et al. High-mobility group box 1 protein plasma concentrations during septic shock. Intensive Care Med. 2007;33(8) 1347-1353.
18. Karakike E, Adami ME, Lada M, et al. Late peaks of HMGB1 and sepsis outcome: evidence for synergy with chronic inflammatory disorders. Shock. 2019;52:334-339.
19. Yasuda T, Ueda T, Takeyama Y, et al. Significant increase of serum high-mobility group box chromosomal protein 1 levels in patients with severe acute pancreatitis. Pancreas. 2006;33:359-363.
20. Charoensup J, Sermswan RW, Paeyao A, et al. High HMGB1 level is associated with poor outcome of septicemic melioidosis. Int. J. Infect. Dis. 2016;2:111-116.

21. Tang Z, Jiang M, Ou-Yang Z, et al. High mobility group box 1 protein (HMGB1) as biomarker in hypoxia-induced persistent pulmonary hypertension of the newborn: a clinical and in vivo pilot study. Int J Med SCI. 2019; 16:1123-1131
22. Baumbusch MA, Buhimschi CS, Oliver EA, Zhao G, Thung S, Rood K, Buhimschi IA. High Mobility Group-Box 1 (HMGB1) levels are increased in amniotic fluid of women with intra-amniotic inflammation-determined preterm birth, and the source may be the damaged fetal membranes. Cytokine. 2016 ;81:82-7.
23. Nakamura T, Yamada S, Yoshioka T. Measurement of plasma concentration of high mobility group box1 (HMGB1) in early neonates and evaluation of its usefulness. Clin Chim Acta. 2012;413(1-2):237-9.
24. Hebra A, Strange P, Egbert JM, et al. Intracellular cytokine production by fetal and adult monocytes. J Pediatr Surg. 2001;36(9):1321-1326.
25. Schultz C, Rott C, Temming P, et al. Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. Pediatr Res. 2002;51:317-322.
26. Dembinski J, Behrendt D, Reinsberg J, Bartmann P. Endotoxinstimulated production of IL-6 and IL-8 is increased in short-term cultures of whole blood from healthy term neonates. Cytokine. 2002;18(2):116-119.
27. Matoba N, Yu Y, Mestan K, et al. Differential patterns of 27 cord blood immune biomarkers across gestational age. Pediatrics. 2009;123(5):1320-1328.

## Ethics

**Ethics Committee Approval:** The study was approved by Eskişehir Osmangazi University Noninterventional Clinical Research Ethical Committee (Decision no: 13, Date: 30.03.2017).
**Informed Consent:** The authors declared that it was not considered necessary to get consent from the patients because the study was a retrospective data analysis.
**Authorship Contributions:** Medical Practices: TBK, DG, ÖA, ÖSO, ANT. Design: ÖA, ANT. Data Collection or Processing: TBK, DG Analysis or Interpretation: ÖA, ÖSO. Literature Search: TBK. Writing. TBK, DG, ÖA, ÖSO, ANT.
**Copyright Transfer Form:** Copyright Transfer Form was signed by all authors.
**Peer-review:** Internally peer-reviewed.
**Conflict of Interest:** No conflict of interest was declared by the authors.
**Financial Disclosure:** The authors declared that this study received no financial support.