DOI:10.2478/jbcr-2023-0016

Original Article

# DIAGNOSTIC UTILITY OF ENDOCAN AND INTERLEUKINS FOR LATE-ONSET NEONATAL SEPSIS

**Preslava R. Gatseva**[1, 2]
**Alexander B. Blazhev**[3]
**Zarko Y. Yordanov**[4]
**Victoria G. Atanasova**[1, 2]

[1]*Department of Pediatrics, Medical University – Pleven, Bulgaria*
[2]*Dr Georgi Stranski University Hospital, Pleven, Bulgaria*
[3]*Department of Anatomy, Histology, Cytology and Biology, Medical University – Pleven, Bulgaria*
[4]*Department of Anaesthesiology and Resuscitation, Medical University – Pleven, Bulgaria*

**Corresponding Author:**
Preslava R. Gatseva
*e-mail: preslava_gatseva@abv.bg*

**Received:** August 18,2023
**Revision received:** September 25,2023
**Accepted:** November 16, 2023

## Summary

The aim of this study was to determine the potential of early inflammatory markers to diagnose late-onset neonatal sepsis – interleukin 6 (IL-6), interleukin 8 (IL-8) and endocan (ESM-1), and to compare them with routinely used markers like C-reactive protein (CRP) and procalcitonin (PCT). A prospective (January, 2022 – January, 2023) clinical-epidemiological study was conducted in a third level NICU in Pleven, Bulgaria. Patients with suspected nosocomial infection and healthy controls were tested. A sandwich ELISA method was used to measure the serum concentrations. Sixty newborns with an average gestational age of 29.75±3.61 gestational weeks were included, of which 35% were symptomatic and infected, 33.3% were symptomatic but uninfected, and 31.7% were asymptomatic controls. The mean values of PCT and IL-6 differ significantly in the three groups. For ESM-1, IL-8 and CRP, the difference was statistically insignificant. The best sensitivity (78%) and negative predictive value (84%) was found for IL-6. The introduction into routine practice of indicators such as PCT and IL-6 may provide an opportunity to promptly optimize the diagnostic and therapeutic approach to LOS.

**Keywords:** neonates; late-onset sepsis; inflammatory markers

## Introduction

Neonatal sepsis is a major cause of morbidity and mortality among infants. In developed countries, four of every ten infants with sepsis die or experience long-term neurodevelopmental impairment [1]. It is described as a clinical syndrome that includes systemic signs of infection, circulatory shock and multisystem organ failure [2]. Despite its importance, there is not yet a clear and accepted sepsis definition for neonates, especially preterm infants, because of differences in the physiology and immune system characteristics in this specific population, compared to adults and children [3]. The "gold standard" is the presence of a positive blood culture, which is assumed to be accompanied by concerning clinical sings [4]. But it turns out

to be very rarely positive, even if clinical and laboratory features strongly suggest a systemic infection [5]. Therefore, "clinical" or culture-negative sepsis is often defined by variable signs and abnormal laboratory parameters in practice [3]. The invasive bloodstream infection during the neonatal period, divided into early-onset sepsis (EOS) and late-onset sepsis (LOS) according to the time of occurrence. EOS reflects more commonly ascending maternal infections, whereas LOS is associated with postnatal nosocomial or public environment and develops after the 72nd hour. In recent decades, the incidence of LOS has increased in parallel with the improvement in survival of preterm infants, especially those with very low birth weight (VLBW), indicating the role of hospitalization and life-sustaining medical devices in the pathogenesis [6]. LOS is a part of the broader concept of healthcare-associated infections in hospitalized neonates, including ventilator-associated pneumonia, urinary tract infections, central nervous system infections, skin (wound), and eye infections [7].

The degree of clinical manifestation of LOS can be highly variable, depending on the virulence of the pathogens and their host-defence mechanisms. Initial symptoms are often non-specific and difficult to differentiate from other non-infectious conditions. Routinely used markers of inflammation such as total blood count (TBC), elevated immature-to-total neutrophil ratio (I/T index), C-reactive protein (CRP) and the "gold standard" positive microbiological culture are usually not sufficiently informative in the initial phase of systemic infection and are defined as "late" indicators. This can result in a delay in the identification of affected neonates, late initiation of treatment and near and long term complications. Therefore, there is a great scientific interest in the field of novel early biomarkers of inflammation that can be valuable in the neonatal practice [8].

The aim of this study was to measure the level of early inflammatory markers at the time when neonatal LOS is suspected – interleukin 6 (IL-6), interleukin 8 (IL-8) and endocan (ESM-1). Validation criteria for diagnostic tests were analyzed – sensitivity, specificity, accuracy, positive and negative predictive value. Their diagnostic value was compared with that of C-reactive protein (CRP) and procalcitonin (PCT).

## Patients and Methods

A prospective (January, 2022 – January, 2023) clinical-epidemiological study was conducted at the Clinic of Neonatology of the University Hospital "Dr. Georgi Stransky", Pleven.

Preterm and term neonates with a stay in the neonatal intensive care unit (NICU) exceeding 72 hours were included. The work group consisted of patients with new onset symptoms suspicious for infection. The presence of three clinical and laboratory indicators and at least one risk

**Table 1.** Clinico-laboratory indicators and risk factors, suggestive of acquired in NICU neonatal infection.

| **Clinical signs (new-onset)**: | **Laboratory signs:** | **Risk factors:** |
|---|---|---|
| Rhythm breathing disorders (apnea, dyspnea) | Low/high WBC count | Mechanical ventilation |
| Increased oxygen needs | Thrombocytopenia | Central venous line |
| Increased requirement for respiratory support | Positive prophylactic microbiological testing | Parenteral nutrition |
| Skin color changes (pale/greyish skin) | Hyper/hypoglycemia | Urinary catheter |
| Tachy/bradycardia | Metabolic acidosis | Gastral tube |
| Abdominal distension | – | Postnatal corticosteroids |
| Vomiting | – | Chronic respiratory and heart failure |
| Diarrhea | – | – |
| Decreased motor activity | – | – |
| Depressed consciousness | – | – |
| Irritability, seizures | – | – |
| Bulged fontanelle | – | – |
| Jaundice | – | – |
| Weight loss | – | – |
| Hypothermia/ hyperthermia | – | – |

factor was accepted as including criteria in the symptomatic group with possible sepsis (Table 1). Exclusion criteria were the presence of severe congenital anomalies and early postoperative period.

In order to analyze the reference limits of the indicators, a suitable control Group 0 was selected from asymptomatic patients - newborns in stable general condition with NICU stay >72 h, with possible presence of risk factors. Samples taken were part of the regular check-up blood tests; microbiologically, only fecal probes were tested on a weekly basis.

On the day of suspicion of infection, along with routine septic-screen tests and microbiological samples (blood culture in all patients, tracheal aspirate in intubated patients, gastric aspirate, cerebrospinal fluid sample in patients with neurological symptoms), 1.5-2 ml of venous blood was drawn and blood serum was separated and stored at – 80°C until analysis.

In the following days, after confirmation or exclusion of the diagnosis, two subgroups were formed from the symptomatic patients:

Group 1 – symptomatic, infected patients. LOS was proven, considering the follow-up tests in the next 5 days such as elevated CRP, leukocytosis/leukopenia; dynamics of clinical condition, treatment, outcome. Both diagnoses of clinical sepsis (with negative microbiological result) and septicaemia (with positive microbiological result) were accepted.

Group 2 – symptomatic, uninfected patients. No evidence of inflammatory activity was proven in the next 5 days; the paraclinical tests remained within reference limits and the microbiological probes were negative; other non-infectious condition or disease was diagnosed.

Measurement of IL-6, IL-8, and ESM-1 serum concentrations was carried out at the end of the study period in the laboratory of Medical University of Pleven. Commercial sandwich ELISA (enzyme-linked immunosorbent assay) based kits were used, respectively: Human IL-6 ELISA Kit, Invitrogen Thermo Fisher Scientific Inc; Human IL-8 ELISA Kit, Invitrogen Thermo Fisher Scientific Inc; Human Procalcitonin ELISA, BioVendor LM; Human Endothelial Cell-Specific Molecule 1 ELISA Kit, CUSABIO TECHNOLOGY LLC. The reaction was performed according to the manufacturer's instructions.

The study was approved by the Ethics Committee of Scientific Research of Medical University – Pleven (Approval Code: 708-CENID/01.06.2022) and was conducted in accordance with the Declaration of Helsinki.

Data were entered and processed with the statistical package IBM SPSS Statistics 25.0 and Office 2021 Excel. A significance level rejecting the null hypothesis was taken as $p<0.05$.

## Results

Sixty newborns were included, of whom 42 (70%) were male and 18 (30%) were female. The mean gestational age of the study contingent was 29.75±3.61 gestational weeks (g.w.) ranging between 25 and 40 g.w. The distribution of study groups was as follows: Group 1 (symptomatic, infected) 35%, Group 2 (symptomatic, uninfected) 33.3% and Group 0 (asymptomatic) 31.7%.

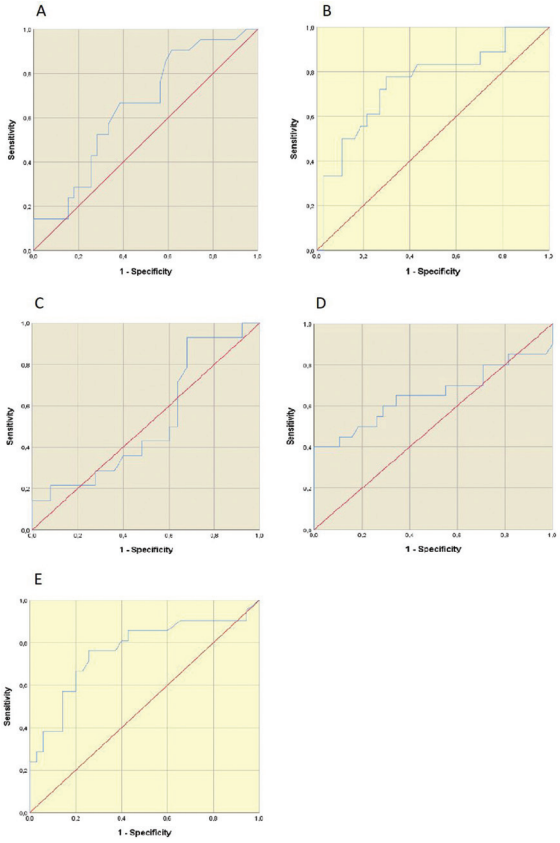


**Figure 1**. ROC curves to determine a threshold value for distinguishing Group 1 from Groups 0 and 2 of: A) ESM-1, area under the curve 0.648, p=0.061 B) IL-6, area under the curve 0.752, p=0.003; C) IL-8, area under the curve 0.523, p=0.815; D) CRP, area under the curve 0.653, p=0.058; E) PCT, area under the curve 0.763, p=0.001.

The comparative analysis of the main groups by the mean values of the markers ESM-1, IL-6 and IL-8, and the indicators CRP and PCT revealed that (Table 2) the three groups significantly differed in only two of the indicators included, namely IL-6 and PCT. In septic neonates from Group 1 compared to non-septic from Group 0 and 2 the mean serum concentration (ng/mL) of IL-6 was significantly higher (30.72±53.48 vs. 29.82±104.02, p=0.015 and 5.07±5.39, p=0.005, respectively). The same correlation was found between the mean PCT serum concentration and the other two groups (2.27±3.22 vs. 0.43±0.57, p=0.004 and 0.44±0.58, p=0.005 respectively).

For PCT and IL-6, Group 2 and 0 mean values were not statistically different from each other. For ESM-1, IL-8 and CRP markers, the difference between the main groups was statistically negligible (p>0.005) (Table 2).

According to the results in Table 2, we could pool the controls with Group 2 (symptomatic uninfected) when searching for threshold values. To determine whether statistically significant threshold values existed for the markers ESM-1, IL-6, IL-8, CRP and PCT, ROC curve analysis was applied, distinguishing Group 1 from Groups 0 and 2. From Figure 1 it is clear that only IL-6 and PCT have significant threshold values, based on the present study calculations (≥27.5 pg/mL for IL-6 and ≥0.46 ng/mL for PCT).

With the established threshold values, an analysis of diagnostic test validation criteria was performed. The results were included in Table 3.

**Table 2.** Comparative analysis of the main groups according to the values of the markers ESM-1 (pg/mL), IL-6 (pg/mL), IL-8 (pg/mL) and the indices CRP (mg/L) and PCT (ng/mL).

| Parameter | Group | N | $\bar{X}$ | SD | P | | |
|---|---|---|---|---|---|---|---|
| | | | | | 0 - 1 | 0 - 2 | 1 - 2 |
| ESM-1 | 0. Controls | 19 | 118.21[a] | 84.00 | 0.130 | 0.967 | 0.092 |
| | 1. Symptomatic infected | 21 | 163.52[a] | 125.11 | | | |
| | 2. Symptomatic uninfected | 20 | 116.00[a] | 85.33 | | | |
| IL-6 | 0. Controls | 17 | 29.81[a] | 104.02 | **0.015** | 0.940 | **0.005** |
| | 1. Symptomatic infected | 18 | 30.72[b] | 53.48 | | | |
| | 2. Symptomatic uninfected | 20 | 5.07[a] | 5.39 | | | |
| IL-8 | 0. Controls | 13 | 45.25[a] | 106.80 | 1.000 | 0.894 | 0.705 |
| | 1. Symptomatic infected | 14 | 141.98[a] | 346.59 | | | |
| | 2. Symptomatic uninfected | 12 | 27.62[a] | 40.20 | | | |
| CRP | 0. Controls | 18 | 3.96[a] | 2.78 | 0.055 | 0.186 | 0.183 |
| | 1. Symptomatic infected | 20 | 21.24[a] | 30.13 | | | |
| | 2. Symptomatic uninfected | 20 | 4.83[a] | 2.52 | | | |
| PCT | 0. Controls | 17 | 0.43[a] | 0.57 | **0.004** | 0.858 | **0.005** |
| | 1. Symptomatic infected | 21 | 2.27[b] | 3.22 | | | |
| | 2. Symptomatic uninfected | 18 | 0.44[a] | 0.58 | | | |

[*]*Identical letters on the verticals indicate the absence of a significant difference, different letters indicate the presence of a significant difference (p<0,05).*

**Table 3.** Threshold values of PCT and IL-6 to distinguish group 1 from groups 0 and 2, and validation criteria values. For PCT, validation criteria are determined according to the threshold value, calculated in the study, as well as according to the reference value of the laboratory.

| Parameter | Threshold value | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Accuracy (%) |
|---|---|---|---|---|---|---|
| **IL-6** | ≥27.5[*] | 78 | 70 | 56 | 87 | 73 |
| **PCT** | ≥0.46[*] | 76 | 74 | 64 | 84 | 75 |
| **PCT** | ≥0.5 | 71 | 74 | 63 | 81 | 73 |

[*]*These threshold values are calculated using data from the present study.*

## Discussion

Late onset neonatal sepsis is defined as an infection occurring after 72 hours after birth. According to the literature, the incidence ranges from 0.6% to 14% of all neonates admitted to hospital [6]. Extremely preterm infants are at the highest risk, with a cited incidence of about 34%. Risk factors for LOS include prematurity, prolonged exposure to invasive procedures, delayed enteral feeding, need for surgical intervention, underlying respiratory and cardiac disease.

The three main challenges in the diagnosis of neonatal sepsis are: the myriad of clinical symptoms that can mimic sepsis; false-negative bacterial cultures in cases of so-called culture-negative sepsis; and the need for empiric treatment for a minimum of 24 to 48 hours while cultures are incubated [9].

An acute phase protein that is very often interpreted simultaneously with complete blood count is C-reactive protein. Usually, it is not outside the reference range in the early stage of infection and as we suggested, it did not reach statistically significant values in our study. The peak of its level is after 24 hours, meaning the sensitivity for neonatal sepsis is lowest in the early stage of infection. It rises in serial testing at the 24-48th hour after the onset of symptoms. The specificity and positive predictive value range from 93 to 100%, so it is referred to as a "specific" but "late" marker for neonatal infection [10].

In contemporary times, there have been considerable scientific studies of diverse serum inflammatory biomarkers, with the primary objective of unveiling a sensitive "early" examination method to evaluate instances of inflammation and infection. These biomarkers encompass PCT, IL-6, IL-8, ESM-1. It is hypothesized that their concentrations exhibit augmentation within the initial hours of an inflammatory response. Demonstrating substantial sensitivity and diagnostic significance, these biomarkers hold potential practical utility. Nonetheless, the outcomes of conducted studies frequently lack conclusive evidence, and the establishment of a uniform methodology remains a challenge in NICUs [11].

Recently, procalcitonin has been more widely used in the diagnosis of neonatal infections. Procalcitonin is a peptide, produced by monocytes and hepatocytes in response to systemic inflammation and, according to some studies, appears to be a more sensitive and earlier biomarker in bacterial infections than CRP [12]. Nonspecific, i.e. infection-independent, induction of PCT synthesis can occur after major surgery, multiple trauma and during the early neonatal period, which is why we excluded these newborns from our study [12]. In neonatal sepsis, its concentrations rise about 4 hours after the proinflammatory effect of bacterial endotoxins and reach their peak after 6-8 hours, thus rising earlier than CRP [13]. Its half-life is 25-30 hours and concentrations are not affected by gestational age. Its elevation is independent of calcitonin and is associated with neurotransmission, immunomodulation, and vascular control during infection and in systemic inflammatory response syndrome (SIRS) [14]. Procalcitonin is usually referred as a highly specific marker for the diagnosis and monitoring of bacterial infections and sepsis, also referring to the severity of the infectious process. In addition, PCT is an indicator of the therapeutic success- a decrease in its plasma levels 24 hours after treatment initiation is connected to favorable therapeutic response [12]. Its diagnostic profile for systemic bacterial infections and necrotizing enterocolitis has shown to be superior to all other acute phase proteins, with a sensitivity and specificity of 87 to 100%. Bustos et al. performed a prospective observational study of a cohort of 53 neonates with clinically suspected late-onset neonatal sepsis. Procalcitonin showed a sensitivity of 88%, a specificity of 71.4%, and a negative predictive value of 87% [15].

In our study, at a threshold value >0.46, PCT showed a sensitivity of 76%, a specificity of 74%, and a negative predictive value of 84%, which are close to those cited in the literature. This proves the necessity to be implemented in the basic septic screening.

Another group of biomarkers that have been evaluated for the diagnosis of neonatal sepsis are the interleukins. Interleukin 6 is an important cytokine of the host's early immune response. A review of studies, conducted from 1990 to 2020 shows that IL-6 is the biomarker that has

been studied more than any other interleukin in newborns [16]. Different threshold values have been proposed, and as this value increases, specificity increases at the expense of sensitivity. In most studies, the results are extremely promising. The mean (30.72) and threshold (≥27.5) steady-state values found in our study are similar to those of Adib et al. [17], who reported sensitivity, specificity, positive and negative predictive values for IL-6 with a threshold value of 30 pg/mL 78%, 95%, 100% and 87% respectively for the diagnosis of neonatal sepsis. For PCT and IL-6, patients in group 1 have a significantly higher mean compared to the other two groups, whose means are not statistically different from each other.

The obtained results demonstrate comparable sensitivity and negative predictive value, albeit at the cost of reduced specificity and negative predictive value. Among the various novel markers investigated in this study, interleukin-6 (IL-6) exhibited the most favorable sensitivity (78%) and negative predictive value (84%).

In the early stage of neonatal bacterial infection, interleukin-8 levels are also increased. In a study by Boskabadi et al., they showed that serum IL-8 concentration in infants with confirmed sepsis was significantly higher than in healthy infants before blood culture positivity [17]. Also, the serum level of this marker in deceased infants with sepsis was much higher than that of surviving infants. The sensitivity, specificity, positive predictive value and negative predictive value for IL-8 were 95%,10%, 97% and 10% respectively, and for CRP was 83%, 86%, 83% and 69% respectively. The cutoff value of IL8 was above 60 pg/mL [15, 17].

In the group of symptomatic infected infants, the mean IL-8 value was 141.98 pg/mL. In the present study, the serum concentrations of the marker, measured by the described methodology, varied within a very wide range, both in the demonstrably ill and in the other two groups of uninfected children. The comparative analysis between the three main groups on the IL-8 value proved that the difference was statistically insignificant.

We also find unsatisfactory results for endocan. This is a new biomarker, being studied in terms of late neonatal sepsis. The mean value in symptomatic infected patients was 163.52 ng/mL. The level in all patients was relatively low and without large variations, compared to results from other authors [18]. The difference between the three groups was not statistically significant and no significant threshold value could be found. It could not be interpreted in terms of diagnostic marker validation criteria.

Our results for endocan are not consistent with those found in the literature, but reports to date are scarce. It is a proteoglycan, secreted by endothelial cells and is suggested to play a role in the pathogenesis of sepsis. An increase in its expression leads to endothelial activation and neovascularization, which are prominent pathophysiological changes associated with inflammation [19].

In a study by Buyuktiryaki et al. (2019), CRP, IL-6, and endocan levels were measured in a total of 102 preterm infants [20]. Overall, while all three biomarkers showed "good performance" in differentiating between sepsis and healthy controls, area under the curve (AUC) values in the groups with proven sepsis showed a more significant value for endocan. Further, serial measurements showed that there was no significant difference in CRP and IL-6 levels between the proven and presumed sepsis groups, while endocan levels were significantly higher. Nonetheless, the endocan was found to show a specificity of 94% and a sensitivity of 94.2% [20, 21].

## Conclusions

The introduction into the routine practice of inflammatory biomarkers such as PCT and IL-6 may provide an opportunity for timely diagnosis, optimization of the therapeutic approach, and reduction of complications from nosocomial infections. According to our results, ESM-1 and IL-8 are not reliable markers of late neonatal sepsis. More studies are needed on a larger cohort of newborns as well as on other early biomarkers of inflammation.

## Acknowledgments

The study received funding from Medical University Pleven, Project № D3/2022.

## References


1. Brocklehurs P, Farrell B, King A, Juszczak E, Darlow B, Haque K, et al. Treatment of neonatal sepsis with intravenous immune globulin. N Engl J Med. 2011;365(13):1201-11.
2. Ershad M, Mostafa A, Dela Cruz M, Vearrier D. Neonatal sepsis. Curr Emerg Hosp Med Rep. 2019;7(3):83-90.
3. Wynn J. Defining Neonatal Sepsis. Curr Opin Pediatr. 2016;28(2):135-40.
4. Wynn JL, Wong HR, Shanley TP, Bizzarro MJ, Saiman L, Polin RA. Time for a neonatal-specific consensus definition for sepsis. Pediatr Crit Care Med. 2014;15(6):523-8.
5. Hornik CP, Fort P, Clark RH, Watt K, Bendjamin DK Jr, Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88(2):69-74.
6. Dong Y, Speer CP. Late-onset neonatal sepsis: Recent developments. Arch Dis Child Fetal Neonatal Ed. 2015;100(3):257-63.
7. Hooven TA, Polin RA. Healthcare-associated infections in the hospitalized neonate: a review. Early Hum Dev. 2014;90(1):4-6.
8. Ng P. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed. 2004;89(3):229-35.
9. Cantey JB, Lee JH. Biomarkers for the Diagnosis of Neonatal Sepsis. Clin Perinatol. 2021;48(2):215-27.
10. Shah BA, Padbury JF. Neonatal sepsis an old problem with new insights. Virulence. 2014;5(1):163-71.
11. Gude SS, Peddi NC, Vuppalapati S, Venu Gopal S, Marasandra Ramesh H, Gude SS. Biomarkers of Neonatal Sepsis: From Being Mere Numbers to Becoming Guiding Diagnostics. Cureus. 2022;14(3):e23215.
12. Atanasova V, Rosmanova R, Andreeva H. Procalcitonin: An innovative marker for bacterial infections. Biochemical, immunological and clinical aspects (chapter 1). Clinical application of immunological investigations. Int Immunol J Clin. 2002;3:153.
13. Ng PC, Lam HS. Biomarkers for Late-Onset Neonatal Sepsis: Cytokines and Beyond. Clinics in Perinatology. 2010; 37(3):599-610.
14. Mitchell, E. Serum Calcitonin Gene-Related Peptide Concentrations in the Horse and Their Relationship to the Systemic Inflammatory Response. Master's Thesis, Virginia Tech, Blacksburg, VA, USA, 2006.
15. Raúl Bustos B, Araneda CH. Procalcitonina para el diagnóstico de la sepsis tardía en recién nacidos de muy bajo peso de nacimiento. Rev Chil de Infectol. 2012;29(5):511-6.
16. Eichberger J, Resch B. Reliability of interleukin-6 alone and in combination for diagnosis of early onset neonatal sepsis: systematic review. Front Pediatr. 2022;10:840778.
17. Boskabadi H, Zakerihamidi M. Evaluate the diagnosis of neonatal sepsis by measuring interleukins: A systematic review. Pediatr Neonatol. 2018;59(4):329-38.
18. Zonda GI, Zonda R, Cernomaz AT, Paduraru L, Grigoriu BD. Endocan serum concentration in uninfected newborn infants. J Infect Dev Ctries. 2019;13(9):817-22.
19. Bechard D, Meignin V, Scherpereel A, Oudin S, Kervoaze G, Bertheau P, et al. Characterization of the secreted form of endothelial-cell-specific molecule 1 by specific monoclonal antibodies. J Vasc Res. 2000;37(5):417-25.
20. Kali A, Shetty KR. Endocan: A novel circulating proteoglycan. Indian J Pharmacol. 2014;46(6):579-83.
21. Buyuktiryaki M, Tayman C, Okur N, Serkant U, Cakir U, Halil H, et al. Can Endocan Predict Late-Onset Neonatal Sepsis? J Pediatr Infect Dis. 2019;14(3):96-102.