Original Article

Endocan - a potential diagnostic marker for early onset sepsis in neonates

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Abstract
Introduction: Neonatal early onset sepsis assessment is based on the history of pregnancy and delivery and nonspecific clinical signs. None of the biomarkers currently in use for clinical practice has adequate prognostic value, so it is not possible to clearly distinguish neonates with culture-proven sepsis from those with only risk factors or clinical suspicion. Endocan is an endothelial mediator involved in the inflammatory response that is present in low concentrations in the serum of healthy subjects, and in much higher concentrations in patients with SIRS and septic shock. The purpose of this study is to evaluate the utility of serum endocan serum levels as a biomarker for the diagnosis of neonatal early onset sepsis (EOS).
Methodology: Serum endocan concentration was measured in newborns with clinical suspicion of EOS admitted to the Neonatal Intensive Care Unit on day 1, 3 and 7.
Results: Serum endocan levels were significantly increased in septic compared to non-septic neonates in the early stages of sepsis (2.43 ± 0.95 *vs*. 1.77 ± 0.57, $p = 0.004$), continued to rise up to 72 hours from onset and then decreased by the seventh day under treatment.
Conclusions: These results suggest a potential role for endocan as an early marker for diagnosis and follow-up in neonatal EOS. Studies on a larger number of cases are needed in order to establish the practical utility of this molecule as a diagnostic tool for clinical practice.

Key words: newborn; early onset sepsis; endocan; proteoglycan; endothelium; systemic inflammatory response.

Introduction

Neonatal early-onset sepsis (EOS) is defined as an invasive infection arising in the first 72 hours since birth, but actually approximately 90% of the cases occur within the first 24 hours of life [1]. The reported incidence of EOS for premature neonates is about 1% with a mortality rate of up to 30% [2]. Positive blood cultures are the gold standard for the confirmation of sepsis, but time to results usually exceeds 24 hours and reported sensitivity is low [3]. EOS diagnostic assessment is based on the history of pregnancy and delivery and nonspecific clinical signs such as poor feeding, persistent crying or irritability, tachycardia, skin mottling, respiratory distress, apnea, lethargy, hypotension or temperature instability [1,3]. This multitude of potentially suggestive signs and symptoms make EOS diagnosis difficult, thus leading to therapeutic delays. Early initiation of antimicrobial therapy has the potential to decrease the morbidity and mortality [3], but given the inherent subjectivity of clinical elements, there is a continuous search for new objective methods of early diagnosis.

Endocan (endothelial cell-specific molecule-1), a proteoglycan expressed by lung and kidney endothelial cells, detectable in the blood stream [4,5] might be useful as a biomarker for sepsis diagnosis. In vitro experiments showed that pro-inflammatory cytokines like interleukin (IL)-1β and tumor necrosis factor (TNF)-alpha, up-regulate the endocan messenger RNA and subsequently induce its secretion from endothelial cells. Available data showed correlation between endocan levels and sepsis severity for adult patients [6].

The purpose of this study is to investigate a possible role for endocan in the diagnosis of neonatal EOS, thus facilitating timely initiation of treatment and improving the outcome of these critically ill newborns.

Table 1. Clinical elements for study inclusion.

Risk factors	Clinical signs	Clinical and/or biological deterioration during the first 72 hours of life (considered to be due to sepsis)
Rupture of membranes > 18 hours	Temperature instability	Hypotension requiring volume expanders or inotropic support
Chorioamnionitis	Apnoea	Anemia requiring packed red cells transfusion
Maternal fever	Need for supplemental oxygen	Acidosis
Positive cultures from the amniotic fluid	Need for non-invasive/ invasive respiratory support	Necrotizing enterocolitis
Vaginal/urinary tract infections during pregnancy	Tachycardia/bradycardia	Intraventricular hemorrhage
Foul smelling amniotic fluid	Feeding intolerance	

The presence of at least one risk factor and three or more clinical signs was required.

Methodology

Study design

We conducted a prospective study of newborns admitted in the Neonatology Intensive Care Unit of our tertiary care center. Information on the gestational age (GA), weight, gender, mode of delivery, need for resuscitation, Apgar score, risk factors for infection and clinical signs of sepsis were noted. The study included term and preterm infants with GA ranging from 26 to 41 weeks, with postnatal age < 24 hours at admission. Study group inclusion was based on the presence of risk factors and clinical signs (Table 1). Infants with congenital anomalies were excluded. Within the study group, the newborns were divided between septic and non-septic groups. The septic group included newborns with confirmed infection (positive blood culture) and probable infection (negative blood cultures but with clinical and laboratory evidence of sepsis). The suspicion of infection was assessed on admission in all newborns with clinical signs compatible with infection. Patients who were initially admitted with suspected sepsis but in whom the diagnosis of sepsis was not supported by clinical or laboratory findings were ultimately assigned to the non-septic group (Table 2). Within the septic group, 22 infants had severe sepsis according to internationally accepted definitions [7,8]. Antibiotic treatment was initiated on admission for all patients in both the septic and non-septic groups according to existing internal protocols [9,10]. In the non-septic group antibiotics were discontinued based on clinical and laboratory findings after 72 hours, while in patients with clinically diagnosed sepsis or with positive cultures, antibiotic therapy continued for at least 7 days.

The study protocol was approved by the Ethical Committee of the University of Medicine and Pharmacy, and written informed consent was obtained from the parents of the newborns before inclusion in the study. Data were anonymised prior to analysis.

Collection of blood samples

From each newborn included in the study, 1 mL of blood was collected from a peripheral vein on the first day of life, at admission in the neonatal intensive care unit (NICU) at the time of initial laboratory workup before any treatment, and then on days 3 and 7. Serum was immediately isolated and frozen at – 80°C until analysis. The concentration of endocan was determined by a sandwich-type enzyme-linked immunosorbent assay using anti-Endocan monoclonal antibodies (Do It Yourself ELISA Kit H1, Lunginnov, Lille, France). Values are expressed in ng/mL [6].

Table 2. Criteria employed for defining neonatal sepsis.

Confirmed sepsis	≥ 3 sepsis-related clinical signs (see Table 1)
	CRP ≥ 6
	PCT > 0.5 ng/mL
	≥ 2 altered serum parameters*
	Blood culture: positive
Probable sepsis	≥ 3 sepsis-related clinical signs (see Table 1)
	CRP ≥ 6
	PCT > 0.5 ng/mL
	≥ 2 altered serum parameters*
	Blood culture: negative
Possible sepsis	< 3 sepsis-related clinical signs (see Table 1)
	CRP < 6
	PCT ≤ 0.5 ng/mL
	< 2 altered serum parameters*
	Blood culture: negative
No sepsis	No sepsis-related clinical signs*
	CRP < 6
	PCT < 0.5 ng/mL
	No altered serum parameters
	Blood culture: negative

*Serum parameters other than CRP or PCT: white blood cells count; absolute neutrophil count, platelet count.

Table 3. Stratification of the newborns included in the study group based on the severity of sepsis.

	Sex	Gestational age		Total
		Preterm	**Term**	
	Female	3	4	7
Non-septic	Male	5	15	20
	Total	**8**	**19**	**27**
	Female	5	1	6
Sepsis	Male	2	2	4
	Total	**7**	**3**	**10**
	Female	5	0	5
Severe sepsis	Male	15	2	17
	Total	**20**	**2**	**22**
Total		**35**	**24**	**59**

Table 4. Clinical and demographic characteristics of the newborns in the study group.

Group	Non-septic	Septic
No	27	32
Gestational age	36.5 ± 3.0	31.5 ± 4.3
Preterm/term	8/19	27/5
Sex (female/male)	7/20	11/21
Birth weight (g)	2812.9 ± 780.2	1795.3 ± 955.8
Delivery (vaginal/c-section)	10/17	13/19
Apgar 1 minute (median) IQR	8 (2)	5 (4)
Apgar 5 minute (median) IQR	8 (2)	7 (3)
Apgar 10 minute (median) IQR	8 (2)	7 (3)

Table 5. Mean serum endocan (ng/mL) in septic versus non-septic neonates measured on days 1, 3 and 7.

Serum endocan (ng/mL) mean ± std. dev.	Group 1 (non-septic)	Group 2 (septic)
Day 1	1.77 ± 0.57	2.43 ± 0.95
p (95% CI of mean difference)		**0.004 (**0.22-1.1**)**
Day 3	2.32 ± 0.3	2.92 ± 1.2
p (95% CI of mean difference)		0.3 (0.6-1.8)
Day 7	1.97 ± 0.57	2.04 ± 0.75
p (95% CI of mean difference)		0.8 (0.6-0.8)

Statistical analysis

IBM SPSS Statistics for Windows 20.0 (by IBM Corp.) was used for statistical analysis. Mean differences for normally distributed variables were evaluated using the Student t test (for independent or paired samples, as appropriate). Receiver operating characteristic (ROC) curve was constructed to assess optimal cut-off values for endocan. A p value < 0.05 was considered as statistically significant.

Results

Patients

The study group consisted of 24 term and 35 preterm newborns evaluated on the first day of life for suspicion of sepsis, for which the parents granted their informed consent. According to the criteria already mentioned, 32 newborns were assigned to the septic group (10 with sepsis and 22 with severe sepsis) and 27 were included in the non-septic group (Table 3). Septic patients had lower gestational age (p < 0.01) and lower birth weight (p < 0.01) (Table 4).

The sepsis was confirmed by positive blood culture in 3 newborns. Two infants died from severe sepsis: one at 28 hours since birth and the other on the 7th day of life. Neither had a positive blood culture.

Endocan values in newborns with clinical suspicion of EOS

In septic compared to non-septic neonates the mean serum concentration (ng/mL) of endocan was significantly higher at admission to the NICU on day 1 (Table 5). In both non-septic and septic newborns the mean endocan concentration continued to rise on day 3, but even though the mean serum level was still higher in septic patients, the difference between the two groups was no longer statistically significant. The mean endocan concentration measured on day 7 was lower compared to day 3 in both groups, but the decrease was statistically significant only in septic newborns (2.04 *vs.* 2.92, p = 0.01) (Figure 1).

Mean endocan serum levels measured on the first day of life were significantly higher in neonates with sepsis (2.39 ± 0.82) and severe sepsis (2.45 ± 1.02) compared to those included in the non-septic group (1.77 ± 0.57) (Figure 2A). Mean endocan serum levels measured on the third day of life remained higher in neonates with sepsis (2.53 ± 0.94) and severe sepsis (3.11 ± 1.42) compared to non-septic newborns, but the difference was not statistically significant (Figure 2B).

ROC curve analysis for the utility of endocan in differentiating between septic and non-septic newborns returned an area under the curve (AUC) of 0.73 (p =

Figure 1. Comparison between endocan serum concentration (ng/mL) on day 1, day 3 and day 7 in non-septic and septic newborns.
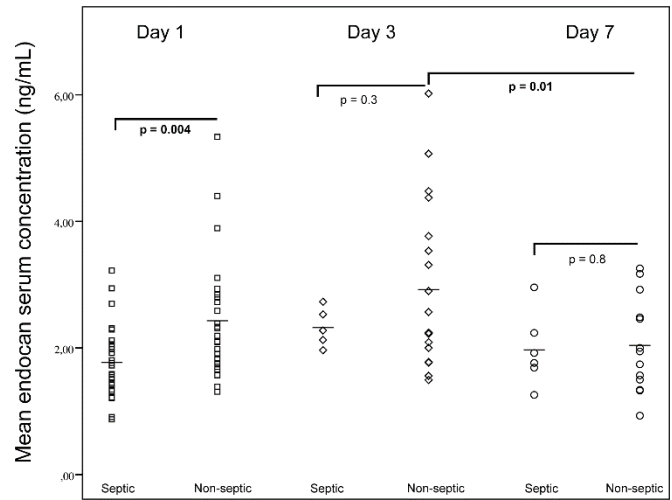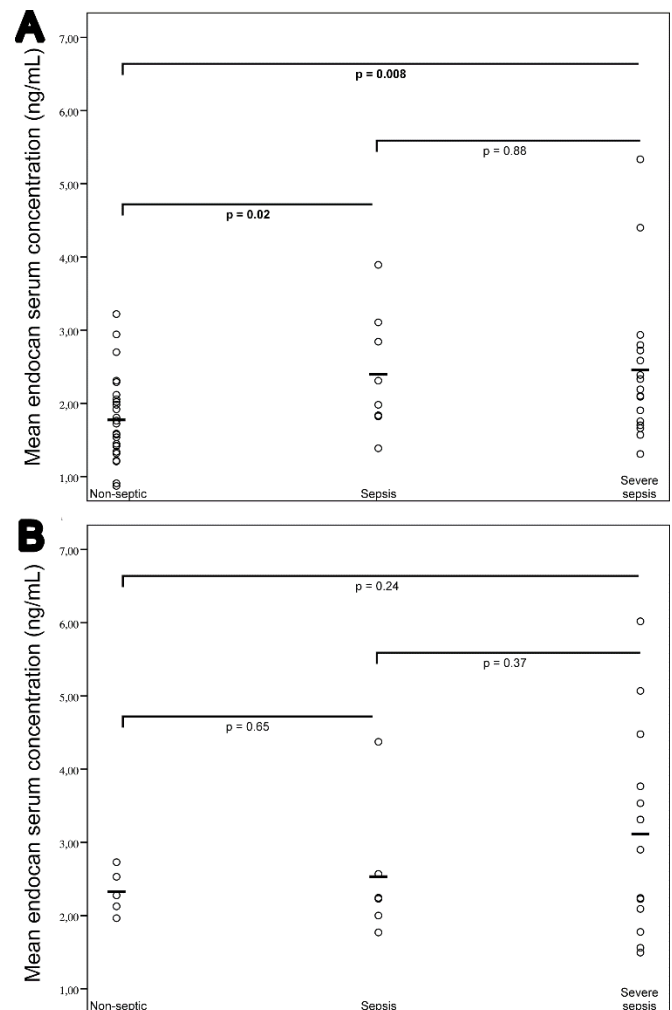

Figure 2. Comparison of mean serum endocan concentrations measured on day 1 (A) and day 3 (B) in newborns with sepsis and severe sepsis versus non-septic neonates.

0.004, 95% CI = 0.597- 0.871) (Figure 3). Based on maximum Youden index, an optimum threshold value of 1.62 ng/mL corresponds to a sensitivity of 88% and a specificity of 50%.

The serum concentration of endocan on the first day of life was statistically correlated with the number of days of mechanical ventilation in non-septic neonates (Pearson correlation coefficient = 0.958, p = 0.01); however, it must be noted that only 5 of 27 patients in this group required mechanical ventilation. We did not find a similar correlation between the mean endocan level and the number of days of mechanical ventilation for newborns with sepsis or severe sepsis. The mean number of days of mechanical ventilation in septic newborns was 9.9 ± 15.5, with a non-normal distribution and a range of 70.

Hypotension was present in 12 infants, 1 with sepsis and 11 with severe sepsis. Inotropic support was necessary for 9 newborns, all preterm infants with severe sepsis, but there was no statistical correlation between the mean serum endocan level and the cardio-vascular status.

Discussion

Currently, there is no test or panel of markers usable for neonatal EOS diagnosis with an acceptable sensitivity and specificity range. It is not possible to clearly distinguish neonates with culture-proven sepsis from those with only risk factors or non-specific clinical signs, and none of the biomarkers currently in use for clinical practice has adequate prognostic value. In critically ill newborns, the decision of initiating antimicrobial therapy is usually formulated on clinical considerations, regardless of the laboratory results.

The vascular endothelium is a component of the innate defense system involved in early recognition and limitation of bacterial invasion. It controls vascular tone and permeability by expression of surface proteins and secretion of soluble mediators, regulates coagulation and thrombosis, and coordinates recruitment and direction of leucocytes towards inflammation sites [5]. The activation of endothelium in the presence of microbial components generates production of cytokines, chemokines and adhesion molecules which attract circulating leukocytes. Activated leukocytes, platelets and endothelial cells lead to the release of vasoactive substances. Excessive endothelial activation may generate vasodilation and organ dysfunction in severe sepsis and septic shock [8].

Endocan is one of the specific endothelial mediators involved in the inflammatory response [11]. It is present in low concentrations in the serum of healthy subjects,

Figure 3. ROC curve analysis for the differentiation between septic and non-septic newborns based on the endocan serum concentration measured on day 1.
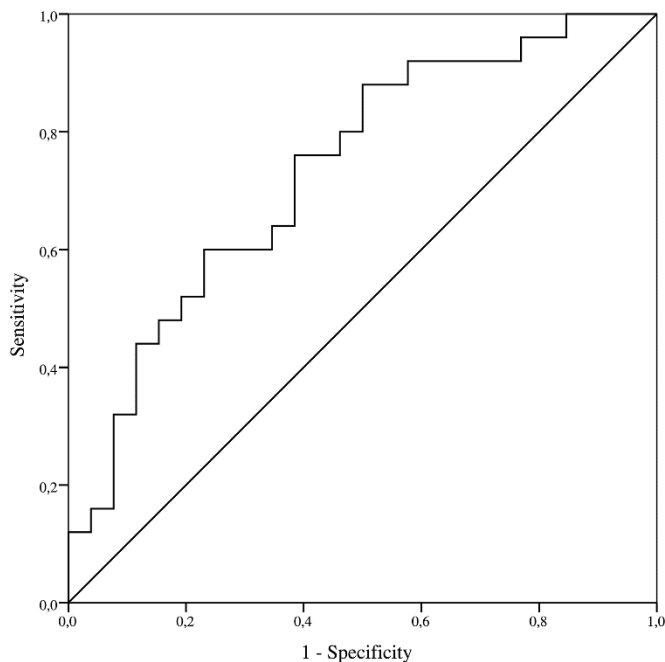
but the levels are much higher in patients with SIRS and septic shock and are correlated with illness severity and prognosis [6,12,13].

Our study shows that septic subjects have significantly higher mean endocan serum concentration than non-septic patients on day 1, which points to endocan as a potential marker for timely diagnosis of neonatal EOS. This is consistent with previously reported data on septic adults [6] and neonates with LOS [14].

When looking at the mean serum levels of endocan reported for adult patients with sepsis (1.95 ng/mL) our data show higher mean concentrations in septic newborns (2.43 ng/mL on day 1 and 2.92 ng/mL on day 3). We determined endocan concentrations on different days and compared both day 1 and day 3 values of endocan in newborns to reported data for adults, taking into account the difficulty in appreciating the exact moment for onset of sepsis in adult patients. The blood was sampled at the time of admission for adults, but this moment most likely does not coincide with day 1 of early-onset neonatal sepsis. Similarly, our data show higher mean endocan concentrations in non-septic newborns (1.77 ng/mL for day 1 and 2.32 ng/mL for day 3) compared to adults with systemic inflammatory response syndrome (0.72 ng/mL).

The significant decrease of serum endocan level on day 7 compared to the concentration measured on day

3 is compatible with the resolution of the systemic inflammatory response following specific treatment, as there are data linking the synthesis and release of endocan to pro-inflammatory cytokines [4,5,11].

The significantly higher level of endocan in septic *vs.* non-septic newborns on day 1 suggests a role of endocan in detection of potential cases of EOS. However, the cut-off value of 1.62 ng/mL has a sensitivity of 88% and a specificity of only 50%, which limits the practical utility of endocan as a single marker for the diagnosis of neonatal EOS. Using a higher threshold value (> 2.15 ng/mL) improves specificity to 81%, but decreases the sensitivity to 52%. For diagnostic purposes, a marker should have a very high sensitivity (approaching 100%) and good specificity (> 85%) [15], or if it is unable to satisfy both criteria, then the optimal cut-off should be chosen so that both the sensitivity and the specificity approach 80% [16]. In this context, serum endocan could probably be integrated with other inflammatory markers and clinical elements in order to develop a composite diagnostic tool for EOS, or it might prove to be useful at a low threshold for ruling out sepsis.

We also found a correlation between the value of endocan on the first day of life and the number of days of mechanical ventilation in non-septic neonates suggesting that endocan is related to the severity of respiratory disease, but the value of this analysis is limited by the small number of patients in this group that were mechanically ventilated. Among septic patients, we had a few outliers with 21, 37 and 70 days of mechanical ventilation, but in these cases the need for invasive respiratory support was determined also by other factors besides the severity of infection.

Our data does not show a correlation between the mean serum endocan level and the cardiovascular status as reflected by hypotension requiring volume expanders or vasopressor agents, suggesting that endocan may be more related to sepsis itself. Such a correlation would have been expected, as the data published on adult patients suggests a correlation between endocan serum concentration and septic shock. However, in neonates, especially preterm infants, hypotension requiring inotropic medication may reflect the contribution of other factors besides the inflammatory process, such as the decreased contractility due to the immature myocardium, deficient transition from intrauterine to extrauterine circulation, patent ductus arteriosus, perinatal hypoxia, or decreased systemic blood flow due to increased intrathoracic pressure in infants requiring positive pressure ventilation [17].

One limitation of our study is the low number positive blood cultures, so newborns with probable sepsis were assumed to be similar to those with culture-proven sepsis. Exposure of neonates to maternal antibiotic treatment during labor and delivery and the small blood volumes collected when obtaining blood cultures (0.5-1.0 mL) may have led to decreased ability to diagnose sepsis by blood culture. On the other hand, neonatal sepsis cannot be ruled out solely based on a negative blood culture result [18,19].

Another limitation of our study is the small number of samples which had to be synchronized with the usual blood tests performed according to the internal protocol. In septic newborns the serum concentration of endocan seems to show an accelerated rise from the first day of life, followed by a less marked increase up to the third day of life and then a significant decrease by day 7, probably due to the resolution of the systemic inflammation as a result of antibiotic treatment. However, only 3 values from each infant might be insufficient to accurately characterize the kinetics of endocan in newborns with EOS.

Conclusion

In conclusion, we report that serum endocan levels are significantly increased in septic compared to non-septic neonates in the early stages of sepsis; the levels continue to rise up to 72 hours from the onset of sepsis and then decrease by the seventh day of treatment. This suggests a potential role for endocan serum level as a marker for early diagnosis and follow-up in neonatal EOS. Further studies on a larger number of cases are needed in order to establish the diagnostic role of this molecule in practice.

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Conflict of interests: No conflict of interests is declared.