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Original Article

Endocan serum concentration in uninfected newborn infants

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Abstract

Introduction: Endocan is a specific endothelial mediator involved in the inflammatory response. Its role in the diagnosis of sepsis has been studied in adult patients and late-onset neonatal sepsis. The clinical signs of early-onset sepsis (EOS) are nonspecific and routinely used biomarkers, such as C-reactive protein and procalcitonin, have low sensitivity, specificity and positive predictive value. Endocan could be a potential diagnostic biomarker of EOS, but at present normal range values for this molecule have not been reported. The aim of this study is to establish the normal values range for serum endocan in term and preterm newborns without risk factors for EOS and to characterize the variation pattern of its levels at different postnatal moments.

Methodology: Mean endocan serum concentration (ESC) was measured in term and preterm newborns without clinical suspicion of EOS at different moments from birth.

Results: ESC (ng/mL) in term newborns was 1.74 ± 0.65 on day 1 and 2.02 ± 0.48 on day 3 respectively, (p = 0.09). In preterm newborns ESC (ng/mL) was 2.02 ± 0.49 and 1.97 ± 0.74, (p = 0.81) for day 1 and 3 respectively. ESC was not significantly influenced by sex, mode of delivery, evidence of fetal distress or presence of minor birth trauma.

Conclusions: ESC (ng/mL) between the first and third day of life in either term or preterm infants don't appear to be significantly influenced by factors that are associated with elevation of inflammatory markers, thus using this biomarker for the diagnosis of EOS might reduce the false positive results.

Key words: newborn; early-onset sepsis; endocan; biomarker; endothelium.

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Introduction

The transition from intrauterine to extrauterine life involves major biochemical and physiological changes including an immature immune response [1]. In neonates, the clinical signs of sepsis presenting in the first 72 hours of life (early-onset sepsis, EOS) are mostly nonspecific. Biomarkers that are routinely used to evaluate for sepsis, such as C-reactive protein (CRP) and procalcitonin, have low sensitivity, specificity and positive predictive value [2]. Blood culture remains the gold standard method for sepsis confirmation, but results are usually available 48-72 hours after sampling, which can lead to delay in adequate treatment and negative outcome of newborns with EOS.

Endocan – endothelial cell-specific molecule-1 – is a circulating 50-kDa dermatan sulphate proteoglycan expressed by endothelial cells [3,4]. The synthesis and release of this molecule is stimulated by vascular endothelial growth factor and proinflammatory cytokines like TNF-α and IL-1β, and inhibited by IFNγ [4-8]. Moreover, *in vitro* studies of sepsis showed that endocan can be degraded by neutrophil-released serine proteases [9]. Serum endocan is elevated in patients with sepsis, both adults and neonates, and its level is correlated with disease severity [5,6], reflecting endothelial activation associated with inflammation. Endocan binds to the Leukocyte Function-associated Antigen-1 (LFA-1) integrin onto the surface of lymphocytes, monocytes and Jurkat cells and inhibits LFA-1 interactions with endothelial Inter Cellular Adhesion Molecule-1 (ICAM-1). In this way, it regulates leukocyte migration to the inflammatory sites, as well as adhesion and activation. Thus, endocan acts as a protective molecule, reducing tissue damage by excessive leukocyte diapedesis [7,9-11].

To our knowledge, there are no published data concerning the levels of endocan in healthy neonates, and this may limit its use in clinical practice. This study aimed to (1) establish the normal values for serum endocan during the first three days of life in term and preterm newborns without infection; (2) characterize the variation pattern of endocan serum levels at different postnatal moments and with different factors associated with elevation of inflammatory markers.

Methodology

Study design

We conducted a prospective study on newborns admitted to the Neonatology Department of our tertiary medical center. Out of the neonates admitted during a 10 months period (from June 2015 to March 2016), 65 were included in the study based on the convenience of the main investigators (when they were working regular hours or were on call), when parent consent was obtained. The size of the study group was limited by available funding.

Information on the gestational age (GA), weight, gender, mode of delivery, evidence of fetal distress (affirmed in the presence of meconium-stained amniotic fluid or abnormal fetal heart rate), need for resuscitation, Apgar score, occurrence of minor birth trauma (either one of the following: caput succedaneum, ecchymosis, clavicle fracture and brachial plexus elongation), risk factors for infection and clinical signs of sepsis were noted.

The study included both term and preterm infants with GA ranging from 33 to 41 weeks, recruited on the first day of life, based on the following criteria: term or preterm newborn (with GA ≥ 33 weeks) with successful transition to extrauterine life, on the first day of life, without risk factors for/or clinical suspicion of sepsis.

The exclusion criteria were: presence of risk factors for infection (rupture of membranes > 18 hours, chorioamnionitis, maternal fever, positive cultures from the amniotic fluid, vaginal or urinary tract infections in the mother during pregnancy, foul smelling amniotic fluid) and/or clinical signs of sepsis (temperature instability, apnoea, need for supplemental oxygen, need for non-invasive or invasive respiratory support, tachycardia/bradycardia, feeding intolerance) [12-15]. Infants with congenital anomalies were also excluded.

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.

Collection of blood samples

One milliliter of blood was collected from a peripheral vein of each infant during the first 6 hours of life. A second blood sample was drawn on day 3 of life. Any excess blood collected during phlebotomy for clinically indicated blood tests was separated and the serum saved for analysis.

Sample handling and analysis

Serum was immediately isolated and frozen at –80°C until analysis. Endocan concentration 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 [5].

Statistical analysis

IBM SPSS Statistics for Windows 20.0 (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). A p value < 0.05 was considered as statistically significant. Mann Whitney U test was used for non-normally distributed data.

Results

The study group consisted of 38 term and 27 preterm newborns (with GA ≥ 33 weeks), as presented in Table 1.

Table 1. Clinical and demographic characteristics of the newborns in the study group.

Category	Term	Preterm
Number (%)	38 (58.5%)	27 (41.5%)
GA (weeks), m ± SD	38.3 ± 1.0	34.5 ± 1.1
Sex (female/male)	17/21	18/9
BW (g), m ± SD	3,232 ± 376	2,136 ± 387
Delivery (vaginal/C-section)	21/17	19/8
Apgar 1 min – median (IQR)	9 (1)	7 (2)
Apgar 5 min – median (IQR)	9 (0)	8 (2)
Apgar 10 min – median (IQR)	9 (0)	8 (1)

GA = gestational age; BW = birth weight.

Table 2. Endocan serum concentration in term and preterm newborns on days 1 and 3 of life.

Serum endocan (ng/mL) mean ± SD (range; 95%CI)	n = 22	Term newborns	Std. error	n = 17	Preterm newborns (GA ≥ 33 weeks)	Std. error	p
Day 1		1.74 ± 0.65 (0.48-3.22; CI: 1.49-2.03)	0.13		2.02 ± 0.49 (1.14-3.20; CI: 1.77-2.27)	0.11	†p = 0.11
Day 3		2.02 ± 0.48 (1.16-2.95; CI: 1.81-2.24)	0.10		1.97 ± 0.74 (0.77-3.40; CI: 1.59-2.35)	0.18	‡p = 0.79
		*p = 0.09			§p = 0.81		

GA = gestational age; *significance coefficient for serum endocan between day 1 and day 3 in term newborns; §significance coefficient for serum endocan between day 1 and day 3 in preterm newborns; †significance coefficient for serum endocan on day 1 between term and preterm newborns; ‡significance coefficient for serum endocan on day 3 between term and preterm newborns.

Following processing of the samples, valid endocan results were obtained for 57 samples on day 1 and 47 samples for day 3. For the rest, the remaining amount of serum was insufficient for the analysis. Out of 38 term infants, there were 36 samples for day 1 and 24 for day 3 to be analyzed; meanwhile, out of 27 preterm infants only 21 samples for day 1 and 23 for day 3 were available for assay. The statistical comparison was possible for 39 sample pairs. In order to establish a trend and to compare the serum levels of endocan on days 1 and 3 for patients included in the study group, we performed the statistical comparison only on these samples.

There were no statistically significant differences of endocan level between the first and the third day of life in either term (p = 0.09) or preterm (p = 0.81) infants, as detailed in Table 2. Also, there were no statistically significant differences of endocan levels between term and preterm infants for either day 1 (p = 0.11) or day 3 (p = 0.79) measurements.

By stratifying the study group using gestational age – week based, we identified a potential downward trend after 39 weeks (Figure 1) for day 1 endocan concentration – significantly higher among neonates born up to 38 weeks compared to those born at 39 weeks and after, 1.94 *vs.* 1.46 ng/mL (p = 0.007). There was no difference in endocan levels on day 3 between groups stratified by the 39 weeks threshold 2.10 ± 0.7 *vs.* 2.10 ± 0.82 ng/mL.

Infants delivered by C-section did not have significantly different concentrations of endocan compared to those born vaginally. Evidence of fetal distress or presence of minor birth trauma did not influence significantly endocan serum levels, as shown in Table 3.

Discussion

The diagnosis of EOS is challenging due to the lack of more specific biomarkers, as many infants present some risk factors or have an intrauterine infection and subsequent inflammation [16]. Because the clinical

Figure 1. Endocan serum concentration (ng/mL) variation with gestational age on day 1.

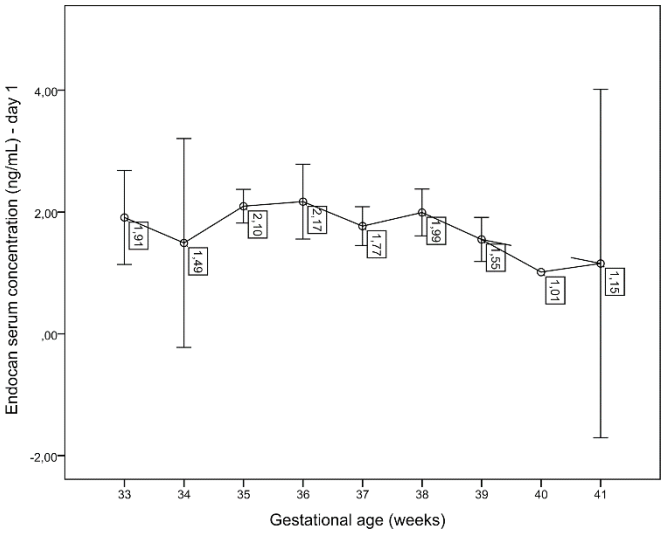

Table 3. Endocan serum concentration (ng/mL) variation with delivery method, fetal distress and minor birth trauma.

Category	Median (IQR) n	Day 1	p	Median (IQR) n	Day 3	p
C-section ♣	31	1.74 (0.64)	0.76	24	1.93 (0.96)	0.25
Vaginal delivery	26	1.83 (0.77)		23	2.26 (0.97)	
Fetal distress	8	1.53 (1.24)	0.43	5	2.27 (0.79)	0.44
No distress	49	1.85 (0.65)		42	2.05 (0.98)	
Minor birth trauma	8	1.98 (0.59)	0.69	6	2.28 (1.15)	0.61
No birth trauma	49	1.79 (0.74)		41	2.02 (0.90)	

signs that may suggest EOS are nonspecific, the diagnosis is mostly based on laboratory findings. Potential biomarkers, such as CRP and procalcitonin (PCT), are extensively used in clinical settings [13,17], but during the first 24 hours of life their specificity and sensitivity are low [18]. For example, CRP may be elevated in multiple other pathological situations besides bacterial infections (viral infections, trauma, ischemic tissue injury, hemolysis, meconium aspiration syndrome or chorioamnionitis without invasive fetal or neonatal disease). Thus, its sensitivity as a biomarker for EOS varies from 29 to 90%. PCT serum levels rise faster than CRP, but the initial reports of sensitivity close to 100% have not been confirmed by subsequent evaluations [15,19,20].

The complex pathophysiological mechanisms of sepsis involve inflammation and endothelial activation as critical determinants of the host response, leading to the hypothesis that biomarkers of endothelium dysfunction might be used as clinical tools in the diagnosis and follow-up of sepsis [21].

There is compelling evidence for including endocan among the tests that may be used to diagnose and predict mortality in adults with sepsis. The first relevant study was conducted by Scherpereel *et al.* [5], who found that endocan level was significantly elevated in adult patients with sepsis and was correlated with sepsis severity and mortality. Evidence available from several studies [6,7] indicates a potential role for endocan as a biomarker for the diagnosis of sepsis in adults with a better discriminative power to distinguish septic patients from non-septic in comparison to CRP and PCT [7,8].

On the other hand, there is precious few data concerning the utility of endocan in neonatal populations. To our best knowledge, there are only two published studies on the value of endocan in the diagnosis of neonatal LOS [11,21]: one that showed that endocan may be used as a marker in order to differentiate LOS *vs.* control in preterm infants (GA ≤ 32 weeks) with specificity, sensitivity, positive predictive value and negative predictive value similar to CRP and IL-6 (using a cutoff value of 9.2 ng/ml). Furthermore, endocan was able to better differentiate between suspected and proven sepsis when compared to CRP, IL-6 and WBC [11]. The other study included late preterm (GA > 34 weeks) and term neonates, and similarly showed a potential diagnostic value for endocan along other new markers such as sTREM and IL-6 in LOS, although with sensitivity and specificity around 70% [21].

The utility of endocan as a marker for early onset sepsis is beginning to be investigated. The starting point should be the characterisation of the physiological values and dinamics for this molecule during the first days of life. The studies mentioned above compare serum endocan concentrations in newborns with sepsis and systemic inflammation, but to the best of our knowledge, there are no published data on the baseline levels of endocan in neonates without infection. Due to the complete absence of data concerning values of endocan in healthy newborns, we compared our measurements of endocan concentrations to those reported in healthy adult patients. The serum levels in newborns were significantly higher compared to adults.

Endocan serum levels for both term (1.74 ± 0.65 ng/mL, mean ± SD) and preterm infants (2.02 ± 0.49 ng/mL) are higher than those reported in the literature for healthy adult volunteers (reported mean value 0.77 ng/mL [0.51-0.95]) [5]. However, our data show lower median concentrations of endocan (ng/mL) in neonates on the first day of life than those reported by Hentschke *et* al. [22] for venous cord blood samples in term newborns (2.91, IQR = [2.20-3.66]). The higher cord blood endocan concentration may mirror the higher endocan levels in maternal plasma observed during the third trimester of pregnancy.

Our data does not show any significant difference in endocan levels between term and preterm infants either on day 1 or day 3. However, our study did not include preterm infants with GA ≤ 32 weeks because neonates in this category that were admitted in our unit during the recruitment stage of the study had perinatal risk factors for infection or nonspecific clinical signs that may suggest EOS and did not meet the inclusion criteria.

Similarly, we found no statistically significant differences in endocan serum concentration due to differences in sex and delivery method. Similar results were reported by Aksoy *et al.* [23] who did not find significant differences between newborns delivered vaginally compared to those delivered by C-section with spinal anesthesia, which was also the type of anesthesia used for C-section in our study group. Delivery mode and anesthesia were included as potential factors that may influence endocan levels by altering oxygenation and inflammatory status of the newborn [24-26].

Our data show that endocan serum levels are not significantly influenced by the presence of minor birth trauma or fetal distress. Thus, in newborns without infection, in the first three days of life, endocan serum level appears not to be significantly influenced by

obstetrical and fetal factors that are associated with elevation of usual inflammatory markers (CRP, PCT). Corroborating with data supporting the endocan elevation in neonatal LOS, we might infer that endocan level could be valuable as a biomarker for diagnosis of EOS and might reduce the false positive results. However, these data should be interpreted with caution, given the small size of the study group and individual variability of physiological adaptation to extrauterine life.

While there is no difference in endocan concentration between term and preterm neonates in either day 1 or day 3 of life, some influence of the GA on serum endocan cannot be completely ruled out as far as day 1 of life is concerned. The post-hoc analysis revealed that the 39 weeks gestational age threshold might imply a certain degree of maturity of lung development. This hypothesis is validated by the data supporting the optimal timing for elective C-section at 39 weeks (rather than earlier) which shows a lower risk of neonatal respiratory morbidity: transitory tachypnea of the newborn, respiratory distress syndrome, and persistent pulmonary hypertension of the newborn [27-30]. The lack of significant difference on the third day of life is to be expected, considering that the transition to extrauterine life is completed by that time for healthy neonates.

A significant limitation of our study is the small number of samples, which proved difficult to overcome in the clinical setting, where the amount of blood collected is limited and sometimes needed also for other diagnostic tests. In order to minimize possible pain and discomfort to the enrolled newborns, we only took two blood samples for study purposes, one as close as possible to the moment of birth, and the other at 72 hours of life, which marks the threshold of diagnosis for EOS. Only two values might be insufficient to accurately characterize the kinetics of endocan in newborns during the first three days of life.

Conclusion

Our results represent a first attempt to provide a normal range of serum endocan levels in the newborn. This information is anticipated to be useful if endocan proves to have value as a potential marker in neonatal sepsis. Endocan serum levels in neonates during the first 3 days of life are higher in our study group compared to those found in healthy adults, so there is a need for different reference values if this parameter is to be used as a diagnostic tool in EOS. Our study shows no statistically significant variation in endocan level between the first and third day of life in either term or preterm infants; moreover, endocan level does not appear to be significantly influenced by sex, delivery method, or factors associated with elevation of inflammatory markers such as minor birth trauma or fetal distress. This suggests that endocan could be a useful biomarker for diagnosis of EOS. However, more studies on a larger number of newborns are warranted in order to establish more accurate kinetics of endocan during the first 3 days of life and the potential role of this molecule in the diagnosis of EOS. Endocan levels seem to drop significantly after 39 weeks of gestation, and this finding may provide a physiological explanation for the lower respiratory morbidity in newborns delivered by elective C-section at more than 39 completed weeks of gestation.

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