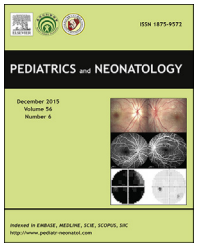
Pediatrics and Neonatology (2015) 56, 415–421

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



ORIGINAL ARTICLE

Endocan and Soluble Triggering Receptor Expressed on Myeloid Cells-1 as Novel Markers for Neonatal Sepsis

Mehmet Saldir [a], Turan Tunc [b,*], Ferhat Cekmez [b], Merih Cetinkaya [c], Tugce Kalayci [c], Kursat Fidanci [a], Oguzhan Babacan [a], Galip Erdem [a], Necmettin Kocak [d], Erkan Sari [a], Emin Ozgur Akgul [e], Mustafa Kul [f]

[a] *Department of Pediatrics, Gulhane Military School of Medicine, Ankara, Turkey*
[b] *Department of Pediatrics, Division of Neonatology, Gulhane Military School of Medicine, Ankara, Turkey*
[c] *Department of Pediatrics, Division of Neonatology, Istanbul Kanuni Sultan Suleyman Teaching Hospital, Istanbul, Turkey*
[d] *Department of Public Health, Gulhane Military School of Medicine, Ankara, Turkey*
[e] *Department of Clinical Biochemistry, Gulhane Military School of Medicine, Ankara, Turkey*
[f] *Department of Pediatrics, Gulhane Haydarpasa Military Hospital, Istanbul, Turkey*

Received May 24, 2014; received in revised form Feb 12, 2015; accepted Mar 17, 2015
Available online 30 April 2015

Key Words
biomarker;
endocan;
neonatal intensive care;
newborn;
sepsis;
sTREM-1

Background: Neonatal sepsis is an important cause of neonatal morbidity and mortality in the neonatal intensive care unit. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) has been evaluated in sepsis and septic shock, and it was found to be valuable in distinguishing septic cases from nonseptic cases. Endocan is constitutively expressed by endothelial cells, and high levels of endocan may be of relevance for the promotion of systemic inflammation. The aim of this study was to investigate whether the levels of sTREM-1 and endocan were increased in late-onset neonatal sepsis.
Methods: Patients were classified into septic and nonseptic groups. Blood was collected from a peripheral vein of all septic newborns and healthy newborns at the time of initial laboratory evaluation before any treatment, and within 48–72 hours after initiation of treatment. Serum sTREM-1 and endocan measurements were performed when the study was finished.
Results: The study population comprised of 50 neonates: 20 nonseptic neonates and 30 septic neonates. The groups were similar with regards to baseline characteristics. The initial measurements of interleukin-6 (IL-6), sTREM-1, endocan, and immature/total neutrophil ratio (I/T ratio) were significantly higher in septic neonates in comparison with nonseptic neonates.

* Corresponding author. Gulhane Military School of Medicine, Department of Pediatrics, Division of Neonatology, 06018 Ankara, Turkey.
E-mail address: ttunc@gata.edu.tr (T. Tunc).

http://dx.doi.org/10.1016/j.pedneo.2015.03.006

Receiver operating characteristic (ROC) curve analyses revealed that IL-6, sTREM-1, endocan, and I/T ratio resulted in significant areas under the curve (AUC) with respect to early identification of septic neonates. Soluble TREM-1 and IL-6 performed best to distinguish septic neonates from nonseptic neonates. Univariate logistic regression analysis showed that increased IL-6 and sTREM-1 were strong predictors of neonatal late-onset sepsis.
Conclusion: Serum sTREM-1, IL-6, endocan levels, and I/T ratio increased in septic neonates. However, the diagnostic accuracy of circulating sTREM-1 seemed to be better than endocan and I/T ratio, but lower than IL-6.

1. Introduction

In spite of advances in neonatal care, neonatal sepsis represents a serious problem in newborns. Late-onset sepsis (LOS) in neonates is defined as infection becoming clinically evident $>$ 72 hours after birth, and it usually appears due to nosocomially acquired organisms.[1] One study revealed an incidence of 0.44% for LOS with a mortality rate of 9%.[2] Of the neonates evaluated for neonatal sepsis, only 3—8% had culture-proven sepsis. This disparity arose from the cautious approach to the management of neonatal sepsis.[3] Because early signs of sepsis in newborns are nonspecific, diagnostic studies are often ordered and treatment initiated in neonates before the presence of sepsis has been proven.[4] Since mortality from untreated sepsis could be as high as 50%, most clinicians believed that the hazard of untreated sepsis did not allow them to wait for confirmation with positive culture results. Therefore, most clinicians initiate treatment in advance.[5]

Inflammation and endothelial activation are critical determinants of the host response and represent an explanation for the complex pathophysiology in sepsis. Accordingly, biomarkers pertaining to inflammation and endothelial activation might be useful in the diagnosis and follow-up of sepsis. Human triggering receptor expressed on myeloid cells-1 (TREM-1) is a 30-kDa glycoprotein belonging to the immunoglobulin superfamily. It plays a considerable role in the innate immune response against invading microorganisms and is selectively expressed in neutrophils and monocytes/macrophages.[6] Expression of TREM-1 is highly upregulated in septic states. Increased levels of TREM trigger the release of proinflammatory cytokines, increase surface expression of cell receptors, and activate neutrophil degranulation. Additionally, expression of TREM-1 is extremely upregulated in septic states and thus is significantly increased in human blood (also known as soluble form sTREM-1).[7] In this context, sTREM-1 has been evaluated in the blood of adults with sepsis, septic shock, and community-acquired pneumonia and it was found to be valuable in distinguishing infected from noninfected patients.[8,9]

Endocan, initially known as endothelial cell-specific molecule-1, is a soluble 50-kDa dermatan sulfate proteoglycan that is constitutively expressed by endothelial cells in lungs and kidneys and can be detected in human blood.[10] Inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, stimulate the upregulation of endocan mRNA and the secretion of endocan from endothelial cells.[11] The secreted form circulates in the human bloodstream and can be detected easily. It is suggested that serum endocan levels were four-fold higher in sepsis patients at intensive care unit admission compared with healthy adult individuals. In the same study serum endocan levels were found to be higher in patients with septic shock than in patients with severe sepsis or sepsis, representing endothelial damage and correlating with the severity of disease and outcome. High levels of endocan have also been detected in patients with cancer.[12] Other studies have described that endocan is not specific for systemic inflammatory diseases. However, high levels of endocan may be of relevance for the promotion of systemic inflammation.[13]

The aim of this study was to investigate whether levels of sTREM-1 and endocan were increased in infected neonates at the first neonatal intensive-care unit (NICU) admission, and to assess their possible value in early diagnosis and follow-up of LOS. We analyzed traditional biomarkers, interleukin-6 (IL-6) and immature/total neutrophil ratio (I/T ratio), as well as sTREM-1 and endocan levels to evaluate septic neonates in NICUs.

2. Methods

2.1. Study design

This prospective study was conducted in two different level III NICUs (Kanuni Sultan Süleyman Teaching and Research Hospital, Ankara, Turkey and Gülhane Military School of Medicine, Istanbul, Turkey). The study protocol was approved by the local Ethical Committee of Gulhane Military School of Medicine and Teaching Hospital and written informed consent was obtained from the first-degree relatives before admission to the study. The study population included late preterm (gestational age of $>$ 34 weeks) and term neonates who were evaluated for LOS during the period of January 2012 to December 2012. Inclusion criteria were postnatal age $\geq$ 72 hours, the presence of nonspecific signs of sepsis (temperature instability, apneic spells, need for supplemented oxygen, need for ventilation,

tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distension, and necrotizing enterocolitis), and clinical deterioration considered to be due to sepsis. Patients who were referred to our center or outpatients with suspected sepsis during the study period were included in the study. Babies born to mothers with clinical chorioamnionitis, babies who were diagnosed with sepsis in the first 72 hours of life, those who used antibiotic treatment for early-onset sepsis, and those who had congenital infections and anomalies were excluded. During the study period a total of 53 patients were evaluated with suspected sepsis. Of these, 23 were excluded from the study because of the criteria mentioned above.

2.2. Diagnosis of infection

Patients were classified according to the criteria reported by Gitto et al[14] They were classified into two groups: septic and nonseptic. The septic group included neonates with proven infection [positive blood or cerebrospinal fluid (CSF) cultures for microorganisms] and probable infection (negative cultures but clinical and laboratory evidence of sepsis: C-reactive protein (CRP) $>$ 5 mg/dL, $\geq$ 3 sepsis-related clinical signs). Patients who were initially hospitalized with suspected sepsis but in whom the diagnosis of sepsis was not supported by clinical or laboratory findings were included as the nonseptic group. All patients in both groups were administered antibiotics initially according to standard protocols. Antibiotic treatments were discontinued in patients without sepsis, according to clinical and laboratory findings, after 48 hours. However, in patients with microbiologically diagnosed or clinically diagnosed sepsis, antibiotic therapies were given for at least 7 days. Patients with negative cultures and those with no clinical/laboratory evidence of infection were classified as the nonseptic group. The likelihood of infection was assessed on admission and laboratory investigations included complete blood count and differential, white blood cell (WBC) and platelet counts, absolute neutrophil count (ANC), I/T ratio, CRP, arterial blood gases, blood, urine, and cerebrospinal fluid (CSF) cultures. Laboratory evidence of infection was positive blood or CSF cultures, metabolic acidosis, leukopenia/leukocytosis, thrombocytopenia, I/T neutrophil ratio $\geq$ 0.2, and CRP $>$ 5 mg/dL. Gestational age, birth weight, gender, survival, microorganisms isolated from blood, CSF cultures, and Apgar score at 1 minute and 5 minutes for all newborns were included in the two groups (septic and nonseptic newborns).

2.3. Blood sampling

Blood was collected from a peripheral vein of all septic newborns and nonseptic newborns at the time of initial laboratory evaluation before any treatment and within 48–72 hours after initiation of treatment. Samples were collected sterile tubes. After centrifugation, serum was kept at −70°C until analysis. Serum sTREM-1 (Human TREM-1 Quantikine ELISA Kit; R&D Systems, Minneapolis, MN, USA) and endocan (a sandwich-type enzyme-linked immunosorbent assay and expressed in ng/mL) measurements were performed when the study was finished.[11]

2.4. Statistical analysis

SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were presented as mean, standard deviation, median [interquartile range (IQR)], frequency, and percent. The distribution characteristics of continuous variables were evaluated with Kolmogorov Smirnov test. Student *t* test was used as a parametric test and Mann–Whitney *U* test was used as a nonparametric test, where appropriate. Paired *t* test or Wilcoxon signed ranks test were used to compare first and second measures in the study group as appropriate. Pearson Chi-square test or Fisher's exact test were used to compare categorical variables as appropriate. Spearman rho correlation test was used to determine the linear association between variables. Receiver operating characteristic (ROC) curve analysis was used to determine the best cut-off points for IL-6, sTREM-1, endocan, and I/T ratio. Then sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated with respect to determined cut-off values. Univariate logistic regression analysis was used to calculate odds ratio (OR) values. A p value $<$ 0.05 was accepted as statistically significant.

3. Results

The study population comprised of 50 neonates: 20 nonseptic patients and 30 septic patients (6 with proven sepsis and 24 with probable sepsis). Demographic characteristics of the patients are shown in Table 1, and the groups were similar with regards to baseline characteristics. Six patients developed positive blood cultures: three of Gram-positive and three of Gram-negative microorganisms. The pathogens of six participants with positive blood culture were as follows: *Staphylococcus aureus* (1), *coagulase-negative staphylococcus* (CoNS) (2), *Klebsiella* spp. (2), and *Escherichia coli* (1). Urine and CSF cultures were negative in all infants. No infant died in either group.

Table 1 Patient demographics.

	Nonseptic group (N = 20)	Septic group (N = 30)	p
Gestational age (wk)	38.3 ± 1.6	37.9 ± 1.7	0.44
Birth weight (g)	3328 ± 383	3201 ± 313	0.20
Male sex	11 (55)	12 (40)	0.30
Maternal fever	5 (25)	8 (27)	0.27
Premature rupture of membranes	4 (20)	5 (17)	1.00
Intrapartum antibiotics	5 (25)	10 (33)	0.53
Cesarean section	6 (30)	12 (40)	0.47
Apgar at 1 min	8.3 ± 0.7	8 ± 0.9	0.22
Apgar at 5 min	9.6 ± 0.5	9.3 ± 0.6	0.16
Umbilical artery pH	7.21 ± 0.08	7.19 ± 0.07	0.38
Age at study entry (h)	93 ± 16	99 ± 24	0.34

Data are presented as *n* (%) or mean ± SD.

Median concentrations of IL-6, sTREM-1, and endocan were significantly higher at the time of sepsis diagnosis (sepsis 1) in the septic group as compared with the nonseptic group. Further, there was a significant difference between the septic and nonseptic groups with respect to the levels of I/T ratio (Table 2). There was a statistically significant difference between the first (sepsis 1) and the second values (sepsis 2) of septic groups' measurements of IL-6 (median = 19, IQR = 11.8–32.5 ng/mL versus median = 5, IQR = 2.0–6.5 ng/mL, $p < 0.001$), sTREM-1 (median = 985, IQR = 576–1400 pg/mL versus median = 836.6, IQR = 702.2–944.8 pg/mL, $p = 0.028$), endocan (median = 29, IQR = 22.5–38.0 ng/mL versus median = 19.7, IQR = 17.2–21.1 ng/mL, $p < 0.001$), and I/T ratio (median = 0.27, IQR = 0.19–0.36 versus median = 0.12, IQR = 0.10–0.16, $p < 0.001$) (Figure 1).

ROC curve analyses revealed that IL-6, sTREM-1, endocan, and I/T ratio resulted in significant areas under the curve (AUC) with respect to early identification of septic neonates. Soluble TREM-1 and IL-6 performed best to distinguish septic neonates from nonseptic neonates. The AUC was 0.97 [95% confidence interval (CI) = 0.931–0.998, $p < 0.001$] for sTREM-1 and 0.96 for IL-6 (95% CI = 0.908–0.998, $p < 0.001$) whereas the AUC was 0.80 for endocan (95% CI = 0.674–0.923, $p < 0.001$) and 0.90 for I/T ratio (95% CI = 0.826–0.987, $p < 0.001$). Table 3 summarizes the optimal cut-off point, sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratio for each biomarker, respectively.

In the next step, taking into account the cut-off values for these four biomarkers, the relationship between biomarkers and sepsis was examined using both univariate and multivariate logistic regression analysis. Univariate logistic regression analysis showed that all variables were associated with sepsis and the probability of sepsis also rose with the increasing values of each biomarkers. According to univariate logistic regression analysis, increased IL-6 and sTREM-1 were strong predictors of neonatal LOS. IL-6 showed the strongest association with neonatal LOS [OR = 266 (95% CI = 22.50–3145.19), $p < 0.001$] followed by sTREM-1 [OR = 126, 95% CI = 16.26–976.27), $p < 0.001$]. ORs for endocan and I/T ratio were found as OR = 7.7, 95% CI = 2.1–27.5, $p = 0.002$; OR = 20.0 (95% CI = 4.7–85.9, $p < 0.001$, respectively; Table 4). Multivariate logistic regression analysis was performed to determine which of these four biomarkers were the independent predictors of sepsis, and IL-6 was found to be the only independent predictor [OR = 157.4 (10.80–2293.54)]. The other three biomarkers were not found to be statistically significant independent predictors of sepsis (Table 4).

4. Discussion

The present study aimed to determine the predictive values and the kinetics of serum sTREM-1, endocan, and other cytokine concentrations in late preterm neonates and term neonates with LOS. Our results revealed that the levels of sTREM-1, endocan, and IL-6 increased significantly in the serum of neonates with LOS, suggesting that sTREM-1 and endocan had a considerable diagnostic performance to diagnose LOS and to monitor the response to therapy thereafter. To the best of our knowledge, this is the first study investigating these two markers simultaneously.

LOS is a relatively frequent event in newborns admitted to NICUs, and it is associated with a high rate of morbidity and mortality.[15] When septic infection occurs, microorganisms invade the blood stream and release various substances that in turn activate the endogenous mediators of the host systemic response. Various molecules such as CRP, procalcitonin, sICAM-1, IL-6, IL-8, TNF-α, mean platelet volume, CD 64 index, soluble e-selectin, p-selectin, and serum amyloid A were found to be higher in the blood of patients with sepsis. Accordingly, they were proposed as markers of sepsis severity, but none gained wide clinical acceptance because they generally reflected a single biological aspect of sepsis, which is a complex and heterogeneous process.[16–18] Recently, sTREM-1 was shown to help identify patients with infection and perhaps also to help predict patients' outcome in many studies.[19] Increased serum sTREM-1 levels have also been found in patients with infectious shock, being closely correlated with the severity of infection.[20]

Many clinical studies conducted on infected newborns revealed that sTREM-1 could be considered to be a valuable biomarker for assessing neonatal infections. Sarafidis et al[19] recently reported elevated sTREM-1 levels in newborns with possible LOS and confirmed LOS. They suggested that sTREM-1 may be used for assessing nosocomial infections in neonates but it was not better than IL-6 in discriminating septic neonates. In our study, increased sTREM-1 levels were strongly associated with LOS as well as IL-6. In addition, studies on critically ill adult patients showed increased plasma sTREM-1 levels in sepsis patients compared with patients without sepsis.[21] Furthermore, in the latter study, sTREM-1 was reported to be better than some commonly used diagnostic biomarkers such as procalcitonin and CRP in detecting sepsis. Nonetheless, it was shown that increased sTREM-1 levels were not specific for infection and could also increase markedly in acute inflammation without infection.

Table 2 IL-6, sTREM-1, endocan levels, and I/T ratio of patients at the first admission.

	Septic group		Nonseptic group		p
	Median	IQR	Median	IQR	
IL-6 (pg/mL)	19.0	11.8–32.5	1.0	0–3.8	< 0.001
sTREM-1(pg/mL)	985	576–1400	73	60–124.5	< 0.001
Endocan (ng/mL)	29	22.5–38	19.8	15.9–24.5	< 0.001
I/T ratio	0.27	0.19–0.36	0.12	0.10–0.16	< 0.001

I/T ratio = immature by total ratio; IL-6 = interleukin 6; sTREM-1 = soluble triggering receptor expressed on myeloid cells-1.

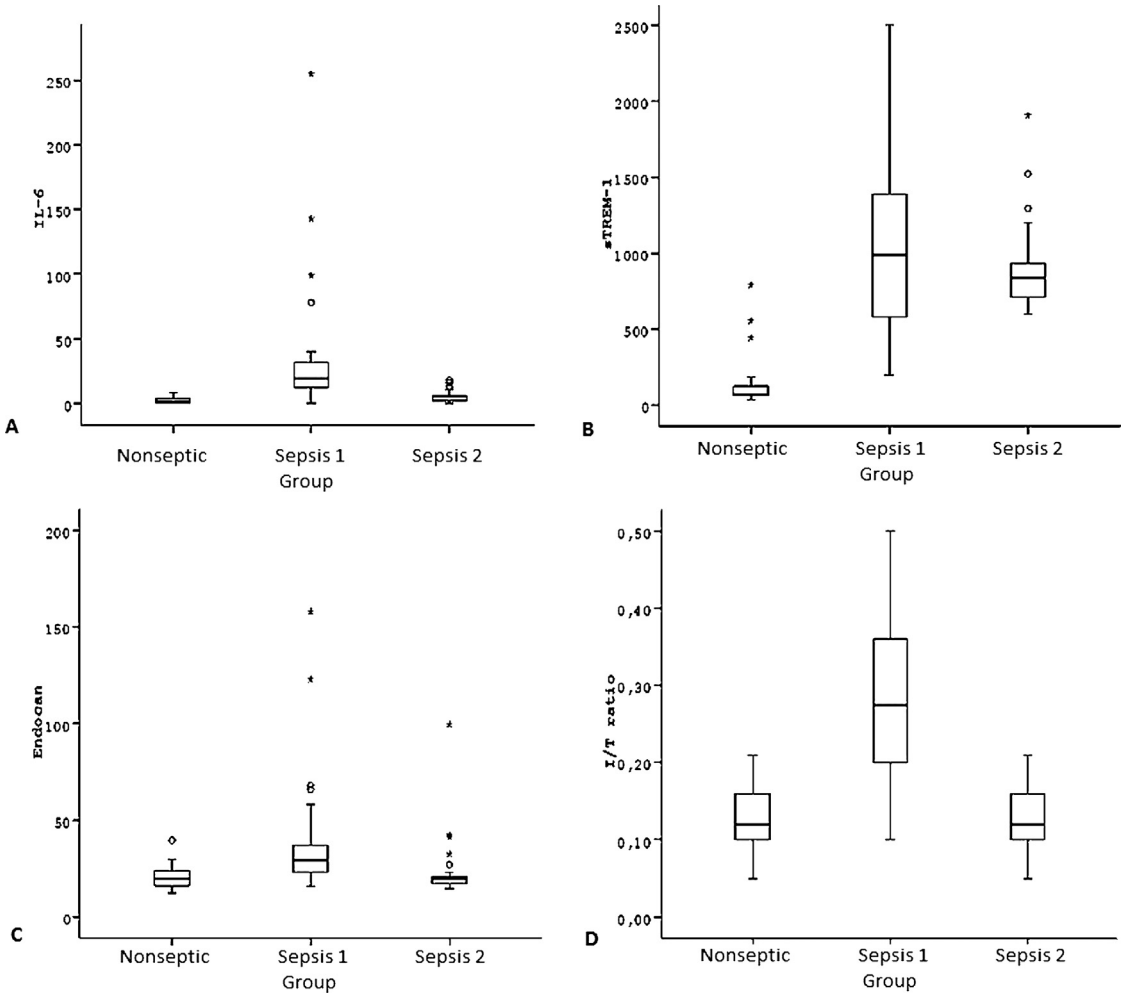


Figure 1 (A)Serum levels of IL-6; (**B**) sTREM-1; (**C**) Endocan; and (**D**) I/T ratio in nonseptic group and septic neonates at the time of diagnosis (Sepsis 1) and 48—72 hours after initiation of treatment (Sepsis 2).

In another study it was shown that within < 24 hours after diagnosis serum levels of sTREM-1 were increased in parallel with sepsis severity, being higher in severe sepsis and septic shock.[22] In a study conducted in mechanically ventilated patients with pneumonia, Gibot et al[21] found higher levels of sTREM-1 in bronchoalveolar lavage fluid samples taken from patients with a diagnosis of community-acquired pneumonia and ventilator-associated pneumonia than from those who did not have pneumonia. In another study of the same group, they sequentially measured plasma sTREM-1 concentrations in septic patients and found that sTREM-1 concentrations remained stable or even increased in nonsurvivors while they decreased in survivors. For this reason, declining plasma sTREM-1 levels could indicate a favorable clinical improvement during sepsis follow-up. In our study, we found a significant decrease in repeat sTREM-1 measurements (within 48—72 hours after initiation of treatment) when compared with the initial

Table 3 Diagnostic accuracy of serum IL-6, sTREM-1, endocan, and I/T ratio in septic neonates.

Diagnostic parameters	Cut-off point	ROC AUC (95%-CI)	*p*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
Biomarkers									
IL-6 (ng/mL)	7	0.96 (0.908—0.998)	< 0.001	93.3	95	96.6	90.5	18.66	0.07
sTREM-1 (pg/mL)	450	0.97 (0.931—0.998)	< 0.001	93.3	90	93.3	90	9.33	0.07
Endocan (ng/mL)	22.5	0.80 (0.674—0.923)	< 0.001	76.7	70	79.3	66.7	2.56	0.33
I/T ratio	0.17	0.90 (0.826—0.987)	< 0.001	83.3	80	86.2	85.7	4.17	0.21

IL-6 = interleukin-6; I/T ratio = immature by total ratio; NLR = negative likelihood ratio; NPV = negative predictive value; PLR = positive likelihood ratio; PPV: positive predictive value; ROC AUC = receiver-operating characteristic area under the curve; sTREM-1 = soluble triggering receptor expressed on myeloid cells-1.

Table 4 Univariate and multivariate logistic regression analysis of diagnostic markers as an indicator of sepsis.

Diagnostic markers	*p*	Unadjusted odds ratio* (95% CI)	Adjusted odds ratio† (95% CI)
IL-6 (< 7 ng/mL)	< 0.001	266.0 (22.50–3145.19)	157.4 (10.80–2293.54)
sTREM-1 (< 450 pg/mL)	< 0.001	126.0 (16.26–976.27)	
Endocan (< 22.5 ng/mL)	0.002	7.7 (2.14–27.49)	
I/T ratio (< 0.17)	< 0.001	20.0 (4.66–85.85)	

* Unadjusted odds ratio: The obtained value with univariate logistic regression analysis.
† Adjusted odds ratio: The obtained value with multivariate logistic regression analysis after the adjustment for other variables (STREM-1, endocan, and I/T ratio).

values (at the 1st admission). We attributed this decline to antibiotic response. Therefore, we also speculate that a progressive decline in levels of sTREM-1 may indicate favorable progress in neonates with LOS.

Endocan is a soluble dermatan sulfate proteoglycan that is expressed and secreted by the vascular endothelium. It has been studied as a novel endothelial cell dysfunction marker in critically ill adults with sepsis, severe sepsis, and septic shock.[23] In a study conducted in ICU, Scherpereel et al[23] demonstrated that endocan blood level was related to the severity of illness and outcome of the patients. In another study, it was shown that an endocan fragment (p14) increased in plasma with severe septic patients, suggesting its likely contribution to the pathogenesis of sepsis.[24] In the present study, we found that endocan levels significantly increased in septic neonates compared with nonseptic neonates. Additionally, within 48–72 hours after treatment, the levels of endocan significantly decreased to normal levels and the difference disappeared in comparison with the nonseptic group.

Our data support the elevation of this molecule in septic neonates. One important point is that the diagnostic value of endocan cannot reach that of sTREM-1. This may be due to the lack of septic shock and also endothelial dysfunction in the study group. As a result, we suggest that sTREM-1 and endocan may be used not only to predict sepsis, but also to follow-up neonates with LOS. The AUC value of CRP was not performed because it was taken into consideration the differential diagnosis of sepsis. Although endocan and I/T ratio were also statistically significant, they were not considered clinically significant compared with IL-6 and sTREM-1 as can be seen from the values of positive and negative likelihood ratio (PLR and NLR; Table 3).

One limitation of this study was the low incidence of culture-proven LOS. Neonates with probable sepsis were assumed to be similar to those with culture-proven sepsis. Furthermore, neonatal sepsis could not be ruled out solely on the basis of a negative blood culture result.[24] Exposure of neonates to maternal antibiotic treatment during delivery increased the rate of false negative blood cultures. In addition, the small blood volumes collected when obtaining blood cultures (0.5–1.0 mL) and the high proportion of low colony count bacteremia result in a diminished sensitivity of blood cultures sepsis diagnosis.[25]

In conclusion, we report that serum sTREM-1 and endocan levels increase in septic neonates and that they both decrease within 48–72 hours of treatment. However, diagnostic accuracy of circulating sTREM-1 seems to be better than endocan but lower than IL-6. Soluble TREM-1 and endocan represent promising novel biomarkers for neonatal sepsis. Large multicentre trials are needed to assess whether determining sTREM-1 and endocan in neonates with LOS can improve the diagnosis and follow-up of infection and reduce unnecessary antibiotic therapy.

Conflicts of interest

There are no conflicts of interest.

Acknowledgments

We thank our colleagues and FAVOR (FMF Arthritis Vasculitis and Orphan disease Research in Pediatric Rheumatology) for their helpful contributions and suggestions. This research received no specific grant from any funding agency in the public, commercial, or not-for- profit sectors.

References

1. Isaacs D, Australasian Study Group for Neonatal Infections. A ten year, multicentre study of coagulase negative staphylococcal infections in Australasian neonatal units. *Arch Dis Child Fetal Neonatal Ed* 2003;**88**:F89–93.
2. Isaacs D, Barfield CP, Grimwood K, McPhee AJ, Minutillo C, Tudehope DI. Systemic bacterial and fungal infections in infants in Australian neonatal units. Australian Study Group for Neonatal Infections. *Med J Aust* 1995;**162**:198–201.
3. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. *N Engl J Med* 2002;**347**:240–7.
4. Kul M, Saldır M, Gülgün M, Kesik V, Sarıcı SÜ, Alpay F. Retrospective evaluation of low birth weight newborns followed up with the diagnosis of respiratory distress syndrome in the newborn intensive care unit. *Gul Med J* 2005;**47**:290–3.
5. van den Hoogen A, Gerards LJ, Verboon-Maciolek MA, Fleer A, Krediet TG. Long-term trends in the epidemiology of neonatal sepsis and antibiotic susceptibility of causative agents. *Neonatology* 2010;**97**:22–8.
6. Bouchon A, Dietrich J, Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J Immunol* 2000;**164**:4991–5.
7. Knapp S, Gibot S, de Vos A, Versteeg HH, Colonna M, van der Poll T. Cutting edge: expression patterns of surface and soluble triggering receptor expressed on myeloid cells-1 in human endotoxemia. *J Immunol* 2004;**173**:7131–4.
8. Routsi C, Giamarellos-Bourboulis EJ, Antonopoulou A, Kollias S, Siasiakou S, Koronaios A, et al. Does soluble triggering receptor

expressed on myeloid cells-1 play any role in the pathogenesis of septic shock? *Clin Exp Immunol* 2005;**142**:62—7.
9. Müller B, Gencay MM, Gibot S, Stolz D, Hunziker L, Tamm M, et al. Circulating levels of soluble triggering receptor expressed on myeloid cells (sTREM)-1 in community-acquired pneumonia. *Crit Care Med* 2007;**35**:990—1.
10. Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. *J Biol Chem* 1996;**271**:20458—64.
11. 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**:417—25.
12. Aitkenhead M, Wang SJ, Nakatsu MN, Mestas J, Heard C, Hughes CC. Identification of endothelial cell genes expressed in an in vitro model of angiogenesis: induction of ESM-1, (beta) ig-h3, and NrCAM. *Microvasc Res* 2002;**63**:159—71.
13. Béchard D, Scherpereel A, Hammad H, Gentina T, Tsicopoulos A, Aumercier M, et al. Human endothelial-cell specific molecule-1 binds directly to the integrin CD11a/CD18 (LFA-1) and blocks binding to intercellular adhesion molecule-1. *J Immunol* 2001;**167**:3099—106.
14. Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, et al. Effects of melatonin treatment in septic newborns. *Pediatr Res* 2001;**50**:756—60.
15. Perez EM, Weisman LE. Novel approaches to the prevention and therapy of neonatal bacterial sepsis. *Clin Perinatol* 1997; **24**:213—29.
16. Berner R, Niemeyer CM, Leititis JU, Funke A, Schwab C, Rau U, et al. Plasma levels and gene expression of granulocyte colony-stimulating factor, tumor necrosis factor-alpha, interleukin (IL)-1beta, IL-6, IL-8, and soluble intercellular adhesion molecule-1 in neonatal early onset sepsis. *Pediat Res* 1998;**44**: 469—77.
17. Cetinkaya M, Ozkan H, Köksal N, Celebi S, Hacimustafaoğlu M. Comparison of serum amyloid A concentrations with those of C-reactive protein and procalcitonin in diagnosis and follow-up of neonatal sepsis in premature infants. *J Perinatol* 2009;**29**: 225—31.
18. Gibot S, Cravoisy A, Kolopp-Sarda MN, Béné MC, Faure G, Bollaert PE, et al. Time-course of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reactive protein plasma concentrations during sepsis. *Crit Care Med* 2005;**33**:792—6.
19. Sarafidis K, Soubasi-Griva V, Piretzi K, Thomaidou A, Agakidou E, Taparkou A, et al. Diagnostic utility of elevated serum soluble triggering receptor expressed on myeloid cells (sTREM)-1 in infected neonates. *Intensive Care Med* 2010;**36**: 864—8.
20. Dimopoulou I, Orfanos SE, Pelekanou A, Kotanidou A, Livaditi O, Augustatou C, et al. Serum of patients with septic shock stimulates the expression of Trem-1 on U937 monocytes. *Inflamm Res* 2009;**58**:127—32.
21. Gibot S, Kolopp-Sarda MN, Béné MC, Cravoisy A, Levy B, Faure GC, et al. Plasma level of a triggering receptor expressed on myeloid cells-1: its diagnostic accuracy in patients with suspected sepsis. *Ann Intern Med* 2004;**141**:9—15.
22. Dimopoulou I, Pelekanou A, Mavrou I, Savva A, Tzanela M, Kotsaki A, et al. Early serum levels of soluble triggering receptor expressed on myeloid cells-1 in septic patients: correlation with monocyte gene expression. *J Crit Care* 2012;**27**: 294—300.
23. Scherpereel A, Depontieu F, Grigoriu B, Cavestri B, Tsicopoulos A, Gentina T, et al. Endocan, a new endothelial marker in human sepsis. *Crit Care Med* 2006;**34**:532—7.
24. Connell TG, Rele M, Cowley D, Buttery JP, Curtis N. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics* 2007;**119**:891—6.
25. Reier-Nilsen T, Farstad T, Nakstad B, Lauvrak V, Steinbakk M. Comparison of broad range 16s rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: a case control study. *BMC Pediatr* 2009;**9**:5.