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Original article

# Performance of interleukin-27 cord blood level as a biomarker predicating early onset neonatal sepsis

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**ABSTRACT**

**Background:** This study designed to evaluate the level of IL-27 in umbilical cord blood and the possibility of its use as a predictor for diagnosis of Early Onset Neonatal Sepsis (EONS). **Methods**: This observational analytic study was conducted by enrolling newborn infants born to pregnant women with antenatal risk factors for sepsis. The infection group that included both suspected and confirmed EONS occurred ≤72 hours after delivery compared to non-infection group. Blood samples were collected from the umbilical artery after cord clamping for biomarkers detection and after 24 hours from venous blood. The concentrations of IL-27 and procalcitonin (PCT) were measured by sandwich ELISA assay. **Results**: A total of 124 neonates were enrolled, 48 (38.7%) neonates were identified in infection group either suspected or confirmed EONS. A significantly higher levels of IL-27 was found in cord blood of babies in the infection group ($p$<0.01). At 24h after birth, the IL-27 continued to show significantly higher levels in infection group ($p$<0.01) compared to non-infection group. The IL-27 showed an increased risk of EONS with an odds ratio of 9.13 and $p$<0.01. The IL-27 through ROC curve analysis showed a better performance in distinguishing neonates with true infection from neonates without infection. A combined performance of IL-27 with PCT in the cord blood and at 24 hours of life showed greater prediction of EONS with $p$ <0.01 for each. **Conclusion**: The IL-27 level in the blood of the umbilical cord may offer a significant predictive tool for EONS either alone or more significantly in combination with PCT.

## Introduction

Neonatal sepsis still considered as one of the major causes of morbidity and mortality during the neonatal period due to high vulnerability of that age group [1]. The functional differences in both innate and adaptive immune responses described in early life comparable to adults, increases the susceptibility to infection in neonates. Neonatal sepsis with the nonspecific nature and late occurring clinical signs often constitutes a challenge for physicians [2]. The disease evolution is known to be rapid, progressive and leads to marked hemodynamic instability with subsequent multi-organ failure. Moreover, if neonates with sepsis can get through the initial inflammatory response, they still liable to several adverse outcomes with extensive neuro-developmental impairment [3]. Primarily, clinicians account on laboratory tests as tools in diagnosis of early onset neonatal sepsis

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(EONS) despite of the reported unreliability of such traditional techniques [4]. Nevertheless, overestimation of EONS seen in some neonatal units contributes to a high antibiotic consumption [5, 6] that may have both short- and long-term adverse consequences [7] and on the other hand, the delay in administration of antibiotic therapy in definitively infected neonates may lead to increased morbidity and mortality [8]. Therefore, the proper diagnosis and an early intervention of neonatal sepsis can potentially improve survival and the rational use of antibiotics will lead to a notable decrease in emerging resistant bacterial strains.

Although the blood culture is considered as the golden standard for diagnosis of bacterial sepsis, the inability to isolate microbial pathogens in the neonatal blood does not exclude EONS [9,10]. The cause behind the high number of cases with negative blood culture is not clear and might be attributed to low levels of bacteria in the blood or to the smaller volume of blood obtained from the sick neonates. Add to that the maternal antimicrobial therapy before or during labor might disguise the detection of bacteraemia in neonates. Also, these cultures have a delay of 48-72 hours to obtain results [11]. Currently, the diagnosis of neonatal sepsis is based on cobbling of clinical assessment together with laboratory biomarkers [12]. Therefore a major sector of neonates are diagnosed as "probable or possible" sepsis but with unidentified bacterial cause [13]; a condition referred to as "culture-negative sepsis" [14].

Assessment of early biomarkers can provide an alternative tool that improves early recognition of EONS. The privilege of using biomarkers in screening for EONS is their ability to find out culture-negative cases that require antimicrobial therapy [15].

Recently, the measurement of different biomarkers in umbilical cord blood can detect the inflammatory response of the fetus started in-utero and used as a diagnostic tool for EONS. Procalcitonin (PCT) remains one of the commonly used biomarkers for diagnosis of EONS, however, the dependence on patient population and the unreliability to distinguish infected from non-infected critically ill patients can lead to variability in its performance [15]. Nevertheless, PCT measurement remains able to differentiate infection from simple colonization in both preterm and full-term neonates despite of such variability in performance [16]. Other biomarkers include tumor necrosis factor-α and interleukins -6 and 8 have been evaluated as promising diagnostic tools for EONS. Presepsin was considered as a biomarker for diagnosis of sepsis in adult, similarly, its level in umbilical cord blood was found to be a reliable predictor for EONS in preterm neonates with premature rupture of membrane [17]. Recently interleukin-27 (IL-27) has been looked at as another candidate biomarker in the serum for diagnosis of sepsis in both adult and children [18-20].

Interleukin-27 is a novel cytokine that was identified in 2002 and classified among the IL-12 family [21]. It is a heterodimeric protein composed of a unique subunit (IL-27p28) and Epstein-Barr virus-induced gene 3 which is shared with IL-35 [22]. IL- 27 is mainly produced by antigen-presenting cells, and expressed in a wide range of cells including placental trophoblast cells [23]. Its biological effects are mediated via IL-27 receptors which is expressed by numerous immune and hematopoietic cells [24]. Although the IL-27 has been described as an essential regulator of inflammation and immune response by multiple studies, its exact role is still controversial [25,26]. Previously, IL-27 has been evaluated as a robust promoter of inflammation. It has been described to promote the initiation of inflammatory cascades, stimulate the expansion of naïve CD4+T cells and the interferon -γ production [21, 27]. More recently, several research have considered IL-27 as a good biomarker to estimate the risk of bacterial sepsis in both critically ill pediatric and adult patients [24, 28, 29].

The aim of our study was to evaluate the level of IL-27 in umbilical cord blood of neonates with EONS and the possibility to use as a predictor for its diagnosis.

## Materials and Methods

### Sample size calculation

Based on the prevalence of EONS in developing countries of 3.5-4.3 case per 1000 live birth, the sample size was derived with a 95% confidence interval and an estimate error of 8%. The calculated total sample size for the study was 125 neonates.

### Participants

An observational analytic study of a cohort was conducted in the neonatal intensive care unit (NICU) at Mansoura University Hospital- Egypt,

over a period of 8 months from May 2022 till December 2022.

We have enrolled all newborn infants born to pregnant women who had one or more of antenatal risk factors for sepsis. These risk factors included maternal fever, prolonged rupture of membranes with leaking amniotic fluid 18 hours or more before delivery, maternal bacterial infection including urinary tract infection or evidence of maternal colonization with group B streptococcus (GBS). Exclusion criteria included pregnant women received antenatal antibiotics for continuous 48hours prior to delivery. A signed informed consent was obtained from the parents/guardians of the enrolled neonates.

We have included 2 groups in our study, case (infection) group and non-infection group that was considered as the control. As the diagnosis of neonatal sepsis is based on cobbling of clinical assessment together with laboratory findings [12], the case (infection) group included either suspected or confirmed infection. The confirmed sepsis cases were considered in the presence of positive neonatal blood culture [9]. The suspected sepsis cases were considered in the presence of negative blood culture associated with clinical signs such as lethargy, poor feeding, abdominal distension, temperature instability, hemodynamic instability, apnea or respiratory distress, increased oxygen requirement or coagulopathy. These clinical signs should be reinforced with ≥2 of abnormal laboratory tests that included absolute neutrophil count less than 7500 or more than 14,500 cells/mm3, low platelet count <150,000 cells/mm3, high absolute band count of >1500 cells/mm3 and ratio of >0.16 of immature to the total neutrophilic count or high C-reactive protein (CRP) [30]. Both suspected and confirmed sepsis occurred ≤72 hours after delivery were counted as cases of EONS and to start antibiotics.

**Biomarker's measurements in blood**

For biomarkers detection, blood samples were collected from the umbilical cord after clamping the umbilical cords before placental delivery. The blood was collected in ethylene diamine tetra-acetic acid-containing tubes from each infant for detection of IL-27, PCT and CRP. After 24 hours of life, blood samples for detection of the same biomarkers were collected again from the newborn infants by venous puncture from a peripheral vein. The collected blood samples were centrifuged at 1000xg for a period of 15 minutes then stored at a temperature of −20°C before analysis.

The concentration of IL-27 was measured by Invitrogen Human IL-27 ELISA Kit (Thermo Fisher Scientific, USA) as per the manufacturers' instructions. It is an enzyme-linked immunosorbent assay (Sandwich ELISA) used for the detection of human IL-27 in a quantitative manner. This technique uses coating capture antibodies (anti-human IL-27) which are adsorbed onto the kit's microwells. In positive cases, after adding the sample, the human IL-27 binds to these coating antibodies. A biotin-conjugated anti-human IL-27 antibody, also referred to as biotin-conjugate, is then added to the wells. This biotin-conjugate will bind to human IL-27 that is already captured by the first coating antibody forming a sandwich like structure. After incubation of the wells, the unbound biotin-conjugate is removed by a washing process. In the following step, Streptavidin-HRP is added to the wells and binds to the bound biotin-conjugate. Following incubation, the unbound Streptavidin-HRP is removed by another washing step. After that, a substrate solution that is reactive with HRP is added into the wells. This will result into the formation of a coloured product with a colour intensity that is directly proportional to the amount of IL-27 in the sample. A human IL-27 standard with a value of 4 ng/mL was diluted to construct seven standard solutions that were used for results interpretation.

The concentration of PCT was measured by Invitrogen Human Procalcitonin ELISA Kit (Thermo Fisher Scientific, USA). This kit utilizes a solid-phase sandwich ELISA assay to quantify the level of human PCT following the same principle as discussed above. A 55 ng/mL human PCT standard was diluted into seven standard solutions which were used for reading the results.

**Neonatal blood culture**

Blood samples were collected aseptically by venous puncture from peripheral veins from all recruited neonates before the administration of antimicrobial treatment. About 1 mL of blood was directly inoculated into a pediatric blood culture bottle and sent instantly to the Medical Microbiology and Immunology Department, Mansoura University, Egypt for subsequent microbiological processing.

The inoculated blood culture bottles were incubated at a temperature of 37°C for seven days and examined daily for evidences of bacterial

growth such as hemolysis, turbidity or gas production. Daily subcultures were performed using appropriate media as MacConkey, blood and chocolate agar plates. Subcultures were repeated daily for one week before considering blood culture as negative.

Identification of the obtained bacterial isolates were conducted by standard microbiological techniques including Gram-stained films, colony morphology and biochemical reactions. API 20E identification kit [bioMerieux] was used to confirm the identification of Gram-negative isolates, while the API® Staph and API® 20 Strep identification kits [bioMerieux] were used to confirm staphylococcal and streptococcal species identification, respectively. For common commensals such as *Staphylococcus epidermidis* (*S. epidermidis*), two positive blood cultures were required to differentiate between contamination and infection.

**Data collection**

In addition to the measurement of the studied biomarkers, all relevant demographic data was collected. These data included gestational age, mode of delivery, gender, presence of maternal fever, maternal use of antibiotics, antenatal steroid or evidence of pre-existing maternal immune suppression.

**Ethical approval**

The institutional research board of faculty of Medicine, Mansoura University approved the research protocol (R.22.03.1667).

**Statistical analysis**

The studied biomarkers were presented in the form of percentages, medians and interquartile ranges. Mann-Whitney U-test was used to compare measured biomarker between studied groups with the SPSS 22.0 software for Windows 10 (SPSS Inc., Chicago, IL, USA). Also, with same software the construction and comparison of receiver operating characteristic (ROC) curves and the respective area under the curve (AUC) were performed. A stepwise multivariate logistic regression model was used to identify variables that independently predicted EONS.

**Results**

During the study period, the umbilical cord blood was collected from 124 neonates where their mothers had one or more antenatal risk factor for infection, 48 (38.7%) neonates were identified to have either suspected or confirmed EONS and considered as infection group.

The suspected EONS with negative blood cultures were 37 (77.1%) while the confirmed EONS with positive blood cultures were identified in 11 (22.9%) babies where 5 showed GBS infection, 3 cases showed *Escherichia coli* infection, 2 cases showed *Enterococcus faecalis* infection, and 1 case showed *S. epidermidis* infection. On the other hand, 76 (61.3%) neonates were included in the non-infection group as they did not fulfill criteria for confirmed or suspected sepsis. **Table 1** showed the basic characteristics of patients included in each group with absence of any significant differences between the two groups.

The comparison of biomarker levels in cord blood between the infection (suspected and confirmed sepsis) and non-infection groups revealed a significantly higher levels of IL-27 in the infection group (1.85 ng/mL vs. 0.38 ng/mL) ($p$<0.01) and same higher significance found for PCT (1.2 ng/mL vs. 0.05 ng/mL) ($p$<0.01). In contrast, cord blood levels of CRP failed to show any significant difference between the two groups (2.1 mg/L vs. 1.8 mg/L) ($p$=0.28) **(Table 2)**.

As the levels of these biomarkers may dramatically change during the early postnatal period, we further analysed the differences in the biomarker levels in the blood 24h after birth between the two studied groups. All biomarker levels showed a difference between the two studied groups as IL-27 was significantly higher in infection group (1.92 ng/mL vs. 0.48 ng/mL) ($p$<0.01), the PCT showed the same higher significance (2.1 ng/mL vs. 0.45 ng/mL) ($p$<0.01), and CRP followed the footsteps of the other biomarkers and raised to a significantly higher level in the infection group (18.7 mg/L vs. 2.8 mg/L) ($p$<0.01) **(Table 2)**.

Considering that these biomarkers levels in the umbilical cord blood may differ with the neonatal gestational age at birth, we further categorized the recruited subjects in the infection group into two subgroups [preterm and term groups]. We could not find any difference in biomarker levels in the cord blood between the preterm and term groups in IL-27 (1.71 ng/mL vs. 1.59 ng/mL) ($p$=0.72), PCT (2.2 ng/mL vs. 2.1 ng/mL) ($p$=0.89), and CRP (2.0 mg/L vs. 1.9mg /L) ($p$=0.88).

Moreover, we further categorized the infection group into different two subgroups based

on positivity of the blood culture [confirmed and suspected groups]. The differences in biomarker levels in the cord blood between both subgroups did not show any significance as follow; IL-27 (1.61 ng/mL vs. 1.48 ng/mL) ($p$=0.56), PCT (2.3 ng/mL vs. 2.1 ng/mL) ($p$=0.77), and CRP (2.3 mg/L vs. 1.88 mg/L) ($p$=0.31).

The independent predictors of EONS among the measured biomarkers in the umbilical cord blood were identified by using a stepwise multivariate logistic regression model. The IL-27 showed an increased risk of EONS with an odds ratio of 9.13 and $p$<0.01. On the other hand, PCT showed rather a lower significance than IL27 with an odds ratio of 1.45 and $p$=0.04. On the contrary, CRP failed to show the same significance with an odds ratio of 1.01 and $p$=0.21 as shown in **table (3)**.

The ROC curve analysis was performed for each studied biomarker in the umbilical cord blood separately as shown in **figure (1)**. The IL-27 showed a better performance in distinguishing neonates with true infection from neonates without infection, with an AUC of 0.749 (0.65-0.89), with a sensitivity of 82.42%, a specificity of 72.25%% and negative predictive value (NPV) of 81.46%. The PCT had AUC of 0.72 (0.62-0.793) with a sensitivity of 78.7%, a specificity of 56.73% and NPV of 74.91%. The cut off values for IL-27 and PCT were 1 ng/mL and 0.05 ng/mL, respectively. The performance of CRP was not significantly predictive for EONS with AUC values of 0.503 (0.414-0.538) and less sensitivity of 54.4% and a specificity of 62.7% **(Table 4)**.

At 24 hours of age, the ROC curve analysis was rerun again for all studied biomarkers individually from the venous blood of included neonates as shown in **figure (1)**. The IL-27 continued to show the same good performance to distinguish neonates with true infection from non-infected neonates with an AUC of 0.729 (0.642-0.821), a sensitivity increased to 88.59%, a specificity of 69.84% and NPV of 81.46%. The PCT showed the same as it had AUC of 0.723 (0.626-0.775), a sensitivity of 86.76%, a specificity of 57.83% and NPV of 87.91%. The CRP after 24 hours of life showed a significant prediction for EONS with AUC values of 0.706 (0.622-0.779), a sensitivity of 67.55%, a specificity of 66.70% and NPV of 69.68% as shown in **table (5)**.

A combined use of both IL-27 and PCT levels in the cord blood showed a greater predictive performance than each one alone with AUC of 0.873 (0.681-0.881), a sensitivity stretched to 96.75% and the same applied for the specificity of 73.92% and the NPV of 95.66%. The same combination continued to perform strongly after 24 hours of life with AUC of 0.881 (0.675-0.903) with a sensitivity increased to 98.68%, a specificity of 74.81% and NPV of 97.45%.

**Table 1.** Demographic and clinical data of the studied groups.

| | **Without EONS (No.76)** | **With EONS (No.48)** | ***p*** |
|---|---|---|---|
| **Maternal age (Ys), median (IQR)** | 26 (19-29) | 25 (20-28) | 0.89 |
| **Antenatal Corticosteroid, n (%)** | 50 (65.8%) | 32 (66.7%) | 0.83 |
| **Antenatal Antibiotics, n (%)** | 33 (43.4%) | 20 (41.7%) | 0.69 |
| **Maternal fever, n (%)** | 22 (28.9%) | 18 (37.5%) | 0.19 |
| **Maternal PROM >18h n (%)** | 16 (21.1%) | 14 (29.2%) | 0.12 |
| **Maternal UTI, n (%)** | 8 (10.5%) | 5 (10.4%) | 0.92 |
| **Maternal GBS colonization, n (%)** | 28 (36.8%) | 22 (45.8%) | 0.08 |
| **Male gender, n (%)** | 37 (48.7%) | 20 (41.7%) | 0.41 |
| **GA at delivery (ws), median (IQR)** | 37.2 (32.5-39.2) | 35.3 (31.1-37.6) | 0.71 |
| **Caesarean section, n (%)** | 32 (42.1%) | 21 (43.8%) | 0.68 |

EONS, early onset neonatal sepsis; PROM, premature rupture of membrane; UTI; urinary tract infection; GBS, group B streptococcus; GA, gestational age.

**Table 2.** Comparison between biomarkers level in infected and non-infected groups at birth and after 24 hours.

| **Biomarkers** | **At birth** | | | **24 hours of life** | | |
|---|---|---|---|---|---|---|
| | **Infection group** | **Non-Infection group** | **P** | **Infection group** | **Non-Infection group** | ***P*** |
| **IL-27 (ng/mL)** | 1.85 | 0.38 | <0.01 | 1.92 | 0.48 | <0.01 |
| **PCT (ng/mL)** | 1.2 | 0.05 | <0.01 | 2.1 | 0.45 | <0.01 |
| **CRP (mg/L)** | 2.1 | 1.8 | 0.28 | 18.7 | 2.8 | <0.01 |

**Table 3.** Stepwise multivariate analysis of ability of clinical variants and biomarkers in cord blood to predict EONS.

| **Variant** | **Multivariate analysis** | |
|---|---|---|
| | **OR (95% CI)** | ***p*** |
| **Gestational age** | 0.97 (0.89-1.06) | 0.56 |
| **Birth weight** | 0.91 (0.29-2.83) | 0.86 |
| **Male gender** | 1.06 (0.11-3.69) | 0.90 |
| **Respiratory support** | 0.961 (0.12-3.21) | 0.91 |
| **IL-27** | 9.13 (6.32-19.8) | <0.01 |
| **PCT** | 1.45 (1.1- 1.63) | 0.04 |
| **CRP** | 1.01 (1.0-1.1) | 0.21 |

**Table 4.** Predictive ability of biomarkers in cord blood to distinguish infected and non- infected groups.

| | **Cut-off** | **Sensitivity** | **Specificity** | **NPV** | **PPV** | **AUC (95% CI)** | ***p*** |
|---|---|---|---|---|---|---|---|
| **IL-27 (ng/mL)** | 1 | 82.42% | 72.25% | 81.46% | 67.25% | 0.749 (0.65-0.89) | <0.01 |
| **PCT (ng/mL)** | 0.05 | 78.7% | 56.73% | 74.91% | 62.87% | 0.72 (0.62-0.793) | <0.01 |
| **CRP (mg/L)** | 5 | 54.4% | 62.7% | 57.63% | 61.12% | 0.503 (0.414-0.538) | 0.189 |
| **IL-27 + PCT** | | 96.75% | 73.92% | 95.66% | 66.42% | 0.873 (0.681-0.881) | <0.01 |

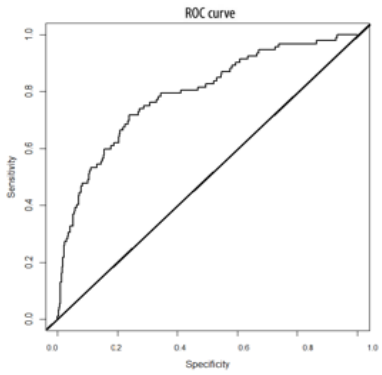
NPV, negative predictive value; PPV, positive predictive value; AUC, area under the curve.

**Table 5.** Predictive ability of biomarkers in blood after 24 hours of life to distinguish infected and non-infected groups.

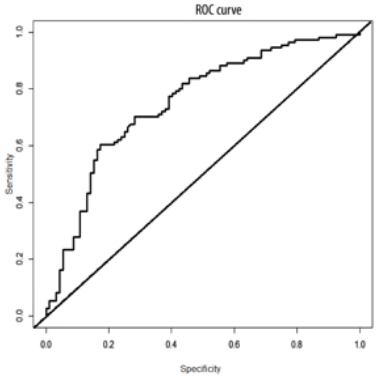
| | **Cut-off** | **Sensitivity** | **Specificity** | **NPV** | **PPV** | **AUC (95% CI)** | ***p*** |
|---|---|---|---|---|---|---|---|
| **IL-27 (ng/mL)** | 1 | 88.59% | 69.84% | 81.46% | 66.51% | 0.729 (0.642-0.821) | <0.01 |
| **PCT (ng/mL)** | 0.05 | 86.76% | 57.83% | 87.91% | 69.83% | 0.723 (0.626-0.775) | <0.01 |
| **CRP** | 5 | 67.55% | 66.70% | 69.68% | 66.10% | 0.706 (0.622-0.779) | 0.04 |
| **IL-27 + PCT** | | 98.68% | 74.81% | 97.45% | 68.21% | 0.881 (0.675-0.903) | <0.01 |

NPV, negative predictive value; PPV, positive predictive value; AUC, area under the curve.

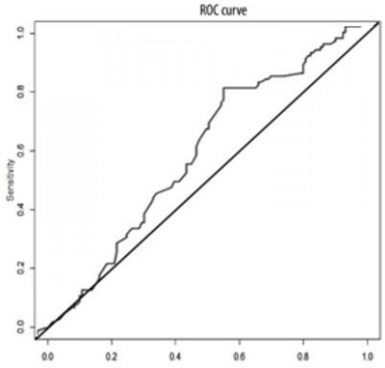
**Figure 1.** Biomarkers level in umbilical cord blood and venous blood after 24 hours of life in neonates with EONS.



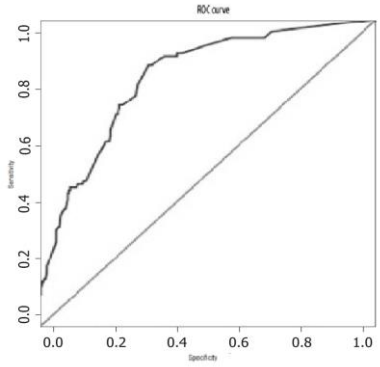

*Umbilical cord blood level of IL27*



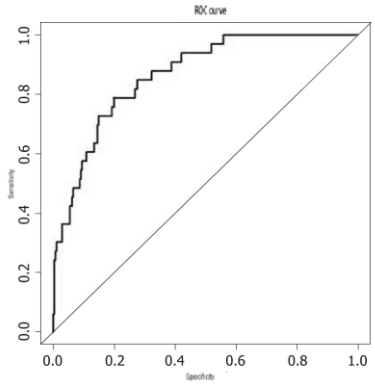

*Umbilical cord blood level of PCT*



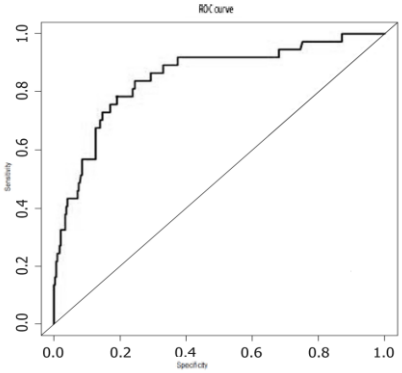

*Umbilical cord blood level of CRP*




*The 24 hours venous blood level of IL27*




*The 24 hours venous blood level of PCT*




*The 24 hours venous blood level of CRP*

**Discussion**

Many biomarkers are considered as potential diagnostic markers for neonatal sepsis. The privilege of using biomarkers in screening for EONS is their ability to find out culture-negative cases that require antimicrobial therapy [16]. Therefore, it is important to rule out sepsis to minimize the number of neonates un-necessarily treated with antibiotics, shorten the period of hospitalization and subsequently lessen the selection pressure for resistant strains [31]. Emergence of different biomarkers measured in cord blood can identify the inflammatory cascade started in-utero and used as a diagnostic tool for EONS. Unfortunately, the available data regards the biomarker dynamics in umbilical cord blood during late pregnancy and at birth are limited.

In our study, we have identified a significantly high level of IL-27 and PCT in cord blood for neonates who developed EONS. The sensitivity of IL-27 in cord blood to distinguish infected from non-infected neonates was slightly higher with AUC 0.749 than that for PCT at AUC 0.72. Additionally, the IL-27 NPV that reflects the ability to rule out EONS was also better than PCT. Nevertheless, the positive predictive values for both biomarkers were 67.25% and 62.87% for IL-27 and PCT, respectively. Such low positive predictive values might be due to the fewer number of positive blood culture reported, along with previously

reported values in neonatal sepsis [11]. Such early prediction offers a very useful diagnostic tool for EONS for newborn babies delivered with inflammatory response initiated in utero. It will improve the patients' prognosis and avoid the unnecessary exposure to antimicrobials in absence of sepsis. However, the level of CRP in cord blood failed to show any significant prediction of EONS between the studied groups.

**Ran and his colleagues** have reported a strong association between the preterm birth and the high levels of IL-27 in peripheral blood as well as the chorion of pregnant women which reflects systemic changes in both mother and fetus [32]. On the other hand, in healthy neonates, **He and his colleagues** have reported lack of any significant elevation of IL-27 in absence of infection through measuring its level in umbilical cord blood obtained from 33 healthy neonates without any risk of infection. The levels of IL-27 continue to lack any significance through the first 72 h after delivery for the same group of patients [33].

All the studied biomarkers were measured again after 24 hours of life from the venous blood of the involved neonates. All the IL-27, PCT and CRP levels have shown significantly higher values in infection group with sensitivity of IL-27 increased to 88.59% that remained slightly higher than 86.76% reported for PCT measured in the same time. The AUC for IL-27 and PCT in babys' blood after 24 hours were 0.729 and 0.723 respectively that still able to distinguish infected from non-infected neonates, nevertheless they were still greater than the AUC reported for the CRP (0.706) which started to be significantly higher in infection group only after 24 hours of life.

Similarly, **Jacobs et al.**, in pediatric patients have reported that IL-27 at a level of > 5 ng/mL considered as a quite specific biomarker for diagnosis of bacterial infection [34]. Moreover, they have reported a low positive predictive value refereed it to the relatively low prevalence of confirmed bacterial infection in pediatric age group.

Our findings were consistent with the allegations of He and his colleagues after measuring a multiplex cytokine profile that showed a higher level of IL-27 in the blood of neonates with either confirmed and suspected EONS when compared to low risk group [33]. Also, **Wong et al.**, who involved critically sick children with multiple sources of sepsis, have reported that IL-27 by itself had specificity and positive predictive value of more than 90% to identify sepsis, with a significantly greater AUC than that of PCT [18]. Moreover, in an experimental study, **Seman and his colleagues** have reported an increase of IL-27 in the circulation of infected neonatal mice that reached more than three-folds following the first day of infection [35].\

Our findings have shown that both negative predictive and to lesser extent the positive predictive values for IL-27 and PCT were markedly higher than CRP. This result is consistent with previous reports which showed that the sensitivity of the CRP was as low as <60% for diagnosis of EONS with premature rupture of membranes that often begins to increase postnatally 12 to 24 hours after the onset of infection [36,37].

On the contrary, **Hanna and his colleagues** have reported that the greater predictive value of IL-27 to distinguish bacterial infection was strictly only to critically ill pediatric patients with positive blood culture with an AUC of 0.75 [38]. In our study, we could not find any significant difference in the IL-27 levels between confirmed and suspected infection as they were both significantly high. Additionally, we could not find any difference in predictive value of IL-27 when gestational age was counted as a variable between preterm and term babies.

The independent predictors of EONS among the measured biomarkers in the umbilical cord blood were identified by using a stepwise multivariate logistic regression model. Both IL-27 and PCT showed strong association with EONS, however there was more than 8 folds prediction with IL-27 with an odds ratio of 9.13 (6.32-19.8, $p<0.01$) compared to 1.45 (1.1- 1.63, $p=0.04$) for PCT. Nevertheless, the CRP did not show any significant association with an odds ratio of 1.01 (1.0-1.1, $p=0.21$). Similarly, He and his colleagues reported that in neonates with sepsis, the venous blood IL-27 have shown the strongest association with an odds ratio of 9.55 followed by PCT with an odds ratio of 1.02 [33].

A use of cord blood IL-27 and PCT combination showed higher sensitivity and predictive achievement than when tested separately. The ROC curve analyses for such combination showed an AUC of 0.873 with sensitivity and NPV increased to 96.75% and 95.66%, respectively. Moreover, after 24 hours of life, the ROC curve analyses for such combination continued to show more positive predictive performance with an AUC of 0.881 and increment of sensitivity and NPV to

98.68% and 97.45%, respectively. Such result indicates that biomarkers can reinforce each other for screening EONS. Same concept has been also proclaimed by **He et al.**, when they combined the predictive ability of both IL-27 and PCT in the venous blood of neonates with EONS where there was an increment of sensitivity to 98.53% and negative predictive value to 97.14% [33].

Interleukin-27 cytokine is released by antigen-presenting cells when they are exposed to inflammatory stimuli and microbial by-products which can explain the elevated levels of IL-27 in EONS reported in our study [19]. In addition, IL-27 acts as a T-cell regulator with reported pro-inflammatory actions through the induction of inflammatory cascades, stimulation of CD4+T cells expansion and the production of interferon -γ [19, 21, 27]. Furthermore, **Wirtz et al.**, studied a murine model of septic peritonitis and reported that IL-27 was quickly induced in this model [39]. They also reported that IL-27 subunit genetic ablation or blocking of IL-27 action by soluble receptor protein was protective in that model [39]. In line with our results, **Wong and his colleagues** have used genome wide-expression analysis and recognized IL-27 as a potential novel biomarker for diagnosis of sepsis [18]. Therefore, it is biologically possible that IL-27 can be used as a biomarker for the diagnosis of sepsis.

The main limitation of our study was the fewer number of confirmed sepsis cases compared to the suspected cases. However, the previously reported incidence for positive blood culture among EONS patients ranges from 2% to 3.3% [40, 41]. Such limitation had a great impact on the positive predictive values of the studied biomarkers. Another limitation to be considered is being a single centre study. Therefore, the predictive role of IL-27 as a diagnostic tool for EONS needs to be validated through an independent multi-centre cohort.

## Conclusions

In conclusion, the IL-27 level in the blood of the umbilical cord may offer a significant predictive tool for EONS either alone or more significantly in combination with PCT. Therefore, an elevated level of IL-27 in cord blood is a promising tool to identify patients with EONS and give guidance for physicians when to start antimicrobials that can reduce the use of unnecessary antibiotics. Our results are to encourage researchers to find more about the role of inflammatory biomarkers in cord blood in order to predict neonatal morbidity.

## Authors' contributions

Wael A. Seleim was responsible for designing the protocol to be carried out, writing the manuscript, clinical evaluation of included study participants besides collecting studied samples and analysis of data. Amira M. Sultan was responsible for writing the manuscript, processing studied samples and analysis of data. Rasha M. Elnagar was responsible for processing studied samples and writing the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

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## Availability of data and material

Will be available on reasonable request.

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## List of abbreviations

EONS: Early onset neonatal sepsis; PCT: Procalcitonin; IL-27: Interleukin-27; S. aureus: Staphylococcus aureus; NICU: Neonatal intensive care unit; GBS: Group B streptococcus; CRP: C-reactive protein; NPV: Negative predictive value; AUC: Area under the curve.

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