Cord blood interleukin-6 as a predictor of early-onset sepsis in preterm babies

Mohamed M. El Bakry[a], Yasser M. Ismail[b], Ahmed A. Sobeih[c] and Ahmed A. Mahmoud[d]

[a]Professor and Head of Pediatrics Department, Faculty of Medicine, Banha University, [b]Professor of Clinical Pathology, Faculty of Medicine, Banha University, [c]Lecturer of Pediatrics, Faculty of Medicine, Banha University, [d]Msc, Faculty of Medicine, Banha University, Egypt

Correspondence to Ahmed A. Mahmoud. Msc, Faculty of Medicine, Banha University, 9276 Mokattam, Cairo, Egypt.
E-mail: dr.ahmedaziem@gmail.com, drahmadata2007@yahoo.com
Mobile number: 00201023710440/01006838956/01144548093

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Background
Despite extensive investigation, no single test meets the criteria that could make it an ideal marker for early diagnosis of sepsis in the newborn. Generally, screening includes a complete blood count with differential white blood cell count and may be accompanied by other adjuvant tests such as evaluation of C-reactive protein.

Objective
The aim of the study was to evaluate the role of cord blood interleukin-6 (IL-6) as an early predictor of early-onset neonatal sepsis in preterm babies.

Patients and methods
We studied 75 preterm babies with risk factors of early-onset sepsis in Benha University Hospitals and measured IL-6 in cord blood samples to correlate them with C-reactive protein, hemoglobin percentage, white blood cells count with immature/total (I/T) ratio, and platelet count. Patients were classified into three groups according to gestational age and subdivided according to sepsis state into proven sepsis group (12 cases), probable sepsis group (48 cases) and noninfected group (15 cases). Samples were taken from the patients early just after birth in the resuscitation room.

Statistical analysis
The collected data were coded, tabulated and statistically analysed using SPSS (Statistical Package for Social Sciences), version 18.0. Results were considered significant if *P* value is less than 0.05 and highly significant if *P* value is less than 0.01.

Results
In our study, cord blood IL-6 was significantly higher in the septic group (probable and proven) with median 371.92 and 124.85 pg/ml, respectively, than in the noninfected group, with median 8.53 pg/ml ($P < 0.0001$). The best cutoff level for IL-6 to diagnose neonatal sepsis is 47.5 pg/ml, with sensitivity 98.3% and specificity 93.3%.

Conclusion
Cord blood IL-6 can be used as a predictor of early-onset neonatal sepsis in preterm babies.

Keywords:
early-onset sepsis, interleukin-6, preterm

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Introduction

Neonatal sepsis is defined as a clinical syndrome of bacteraemia with systemic signs and symptoms of infection in the first 4 weeks of life [1]. Early-onset neonatal sepsis is a serious complication with a mortality rate ranging from 1.5% in term babies to almost 40% in very-low-birth-weight infants [2].

There is no single definition of early-onset sepsis (EOS) in the newborn; various definitions exist, each with subtle differences. EOS refers to an infection of the blood stream proven by culture. EOS is usually acquired vertically from the mother and manifests in the first 24 h to 7 days after birth [3].

The impaired innate immune function of premature infants predisposes them to invasive infections. As the foetal immune response begins at 24 weeks' gestational age and development occurs until reaching term, premature neonates do not benefit from complete immune system development, making them more susceptible to infection from organisms that term infants may be able to suppress [4].

Although a positive blood culture remains the standard for diagnosing neonatal sepsis, many investigators have assessed measuring the host response as an adjunct to culture-based diagnosis. The goal of serum biomarker research is to identify a means by which an infected child can be identified rapidly, before the onset of life-threatening symptoms [5].

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Screening tests, including white blood cell (WBC) count and acute-phase reactants, such as C-reactive protein (CRP), have poor positive predictive values in septic neonates: 40% in symptomatic neonates and as low as 1–2% in asymptomatic neonates [6]. Neonatal blood culture-positive rates have been found to range from 25 to 54% [7].

Interleukin-6 (IL-6) belongs to the family of cytokines. It is one of the mediators of inflammation that are released early in the course of septic shock and is crucial in initiating the immune response, as well as to the activation of T lymphocytes and B lymphocytes and lymphocyte proliferation and differentiation. In addition, IL-6 is a potent pyrogen. It also induces the release of acute-phase proteins like CRP [8].

IL-6 reaches its peak after 2 h of bacterial stimulus onset, so that its level may be elevated before the start of the symptoms and before the rise of routinely used markers [9]. IL-6 was reported to appear earlier in plasma than CRP [10]. Umbilical cord IL-6 is consistently increased in newborns with EOS [11].

The diagnosis of infection in neonates is difficult, because of the nonspecific clinical presentation and the lack of reliable diagnostic tests. As a result of this uncertainty, antimicrobial chemotherapy is often commenced on the slightest clinical suspicion of infection. Recently there has been great interest in the potential diagnostic value of a range of haematological and immunological surrogate markers of infection [12].

In recent years, most studies and reviews have confirmed the role of inflammatory factors such as cytokines – for example, IL-6 and IL-8, in neonatal infection because these markers are released immediately at the beginning of neonatal infection from the liver and immune cells and then show increased levels in the blood. These inflammatory markers have vital roles in most of the inflammatory processes and in immunity of the body against viral and bacterial infections; thus, they function as phagocytic agents for bacteria and viruses [13].

Patients and methods

This cross-sectional study was conducted on 75 preterm babies with risk for sepsis; their gestational ages ranged from 26 to 36 weeks from June 2014 to May 2015.

Study methods

Inclusion criteria

The following were the criteria for inclusion into the study: maternal history of fever; maternal history of resistant urinary tract infection; history of premature rupture of membranes (PROM) or bad odour of amniotic fluid; and history of immune suppressant drug intake. Children from normal vaginal delivery or caesarean section, either single or twins, both male and female, were included.

Exclusion criteria

Full-term babies, those with hypoxic ischaemic encephalopathy, babies with congenital pneumonia, those with congenital syndromes such as Down's syndrome, Turner's syndrome, Edward's syndrome, babies with any congenital heart disease, and infant to diabetic mothers were excluded.

The 75 neonates were classified into three age groups: group 1, comprising infants from 26 to 29 weeks; group 2, comprising infants from 30 to 33 weeks; group 3, comprising infants from 34 to 36 weeks. Each group was classified into three subgroups: infants with proven sepsis, which included infants with a positive culture; infants with probable sepsis, which included those with a negative culture with clinical signs of sepsis and elevated I/T ratio greater than 0.2 or WBC count greater than 5000/mm^3 or greater than 25 000 mm^3 at evaluation; noninfected infants, which included those not fulfilling any criteria for proven or probable sepsis.

Grouping was done before measuring levels of IL-6 to avoid possible bias. Cord blood samples were taken from the 75 newborns and kept frozen at -20°C until the end of the study to be analysed for IL-6. Evaluation of CRP and complete blood count with differential WBC count were also done in a blinded manner. Samples were taken from the patients in the resuscitation room soon after delivery.

Interventions

All neonates in the study were subjected to the following: detailed history taking; careful clinical examination; laboratory investigations including complete blood count with differential WBC count, CRP, blood culture and cord blood IL-6 level by means of the enzyme-linked immunosorbent assay technique (Ani Biotech Oy, Orgenium laboratories, Vantaa, Finland).

Collection of cord blood samples

Two millilitres of blood were obtained from the umbilical cord of each baby by free flow technique using available commercial sets under complete aseptic conditions; blood was left to clot and was then centrifuged for 10 min at 5000 rpm. The sera

were separated and stored at -20°C, avoiding multiple freeze–thaw cycles until the assay of IL-6, which was carried out using the AviBion human IL-6 ELISA kit (Ani Biotech Oy, Orgenium Laboratories).

Statistical analysis

The collected data were coded, tabulated and statistically analysed using SPSS (Statistical Package for Social Sciences), version 18.0 (IBM SPSS Statistics, Thousand Oaks, California, USA). Inferential analyses were performed for quantitative variables using the independent *t*-test in cases of two independent groups with parametric data. Inferential analyses were performed for qualitative data using the χ^2-test for independent variables, whereas correlations were determined using the Pearson correlation for numerical parametric data. Receiver operating curve was used to evaluate the value of different tests between groups. *P* values less than 0.05 were considered significant; otherwise they were not. The *P* value is a statistical measure for the probability that the results observed in a study could have occurred by chance.

Results

Table 1 shows that the frequency of maternal fever is significantly higher in proven EOS with a *P* value less than 0.05.

Table 2 shows that in group 1 CRP was positive in 32% of infants in the proven sepsis group and in 68% of infants in the probable sepsis group, whereas in group 2 CRP was positive in 12.5% of infants in the nonseptic group, in 16.7% of infants in the proven sepsis group and in 70.8% of infants in the probable sepsis group, whereas in group 3 CRP was positive in 20% of infants in the nonseptic group, in 16% of infants in the proven sepsis group and in 64% of infants in the probable sepsis group. The differences were highly significant ($P < 0.001$).

Table 3 shows that IL-6 is highly significantly different in all age groups, being the highest in group 1, with a mean of 249.12 pg/ml, followed by a mean of 134.25 pg/ml in group 2 and a mean of 40.15 pg/ml in group 3.

Table 4 shows that cord blood IL-6 is significantly high in the proven sepsis group, with a mean of

Table 1 Comparison of septic and nonseptic groups regarding risk factors for early-onset sepsis

Risk factors	No [*n* (%)]	Proven [*n* (%)]	Probable [*n* (%)]	Total [*n* (%)]	χ^2	*P* value
Maternal fever						
–	14 (93.3)	1 (8.3)	41 (85.4)	56 (74.7)	28.7	<0.05
+	1 (6.7)	11 (91.7)	7 (14.6)	19 (25.3)		
Maternal UTI						
–	13 (86.7)	11 (91.7)	46 (95.8)	70 (93.3)	2.1	>0.05
+	2 (13.3)	1 (8.3)	2 (4.2)	5 (6.7)		
PROM						
–	3 (20.0)	4 (33.3)	6 (12.5)	13 (17.3)	3.1	>0.05
+	12 (80.0)	8 (66.7)	42 (87.5)	62 (82.7)		
Chorioamnionitis						
–	15 (100.0)	9 (75.0)	47 (97.9)	71 (94.7)	7.04	>0.05
+	0 (0.0)	3 (25.0)	1 (2.1)	4 (5.3)		

PROM, premature rupture of membranes; UTI, urinary tract infection.

Table 2 Comparison between the septic and nonseptic groups regarding C-reactive protein titre

Subgroups	CRP in group 1 [*n* (%)]	CRP in group 2 [*n* (%)]	CRP in group 3 [*n* (%)]	Total [*n* (%)]	χ^2	*P* value
No sepsis	0 (0.0)	3 (12.5)	12 (46.2)	15 (20.0)	23.8	<0.001
Proven sepsis	8 (32.0)	4 (16.7)	0 (0.0)	12 (16.0)		
Probable sepsis	17 (68.0)	17 (70.8)	14 (53.8)	48 (64.0)		
Total	25 (100.0)	24 (100.0)	26 (100.0)	75 (100.0)		

CRP, C-reactive protein.

Table 3 Comparison between different age groups regarding cord blood interleukin-6

Cord blood IL-6	Groups	*N*	Mean	SD	Minimum	Maximum	*F*	*P* value
	26–29 weeks; group 1	25	249.12	116.64	88	487	26.6	<0.001
	30–33 weeks; group 2	24	134.25	120.48	3	400		
	34–36 weeks; group 3	26	40.15	61.54	1	200		

IL-6, interleukin-6.

Table 4 Comparison between the septic groups (probable and proven) and the noninfected group regarding cord blood interleukin-6

Cord blood IL-6	Groups	*N*	Mean	SD	Minimum	Maximum	*F*	*P* value
	No sepsis	15	8.53	1.13	1	5	90.8	<0.001
	Proven sepsis	12	371.92	73.33	302	487		
	Probable sepsis	48	124.85	81.70	10	287		

IL-6, interleukin-6.

371.92 pg/ml, and in the probable sepsis group, with a mean of 124.85 pg/ml, and is very low in the nonseptic group, with a mean of 8.53 pg/ml.

Table 5 shows that IL-6 has highly significant positive correlation with body weight, length of stay, total leucocyte count and I/T ratio.

The receiver operating characteristic curve defines the best cutoff point for cord blood IL-6 to diagnose neonatal sepsis as greater than 47.5 pg/ml with sensitivity 98.3%, specificity 93.3, positive predictive value 98.3% and negative predictive value 93.3% (Table 6).

Table 5 Correlation between cord blood interleukin-6 and different variables

Different variables	*r*	*P* value
GA (weeks)	−0.65	>0.05
Apgar score	−0.17	>0.05
Weight (kg)	−0.60	<0.001
Length of stay (days)	0.95	<0.001
Hb (g/dl)	0.21	>0.05
RBC count	0.22	>0.05
TLC	0.911	<0.001
I/T ratio	0.81	<0.001
Platelet count	−0.38	>0.05
CRP titre	−0.21	>0.05

CRP, C-reactive protein; GA, gestational age; RBC, red blood cell.

Table 6 Receiver operating characteristic curve values for cord blood interleukin-6

AUC	Cutoff	Sensitivity	Specificity	PPV	NPV
0.9	47.5	98.3	93.3	98.3	93.3

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

Discussion

Despite improved neonatal care over the past decades, infections remain common and sometimes life threatening in neonates admitted to the neonatal ICU [14]. Early recognition and diagnosis of neonatal sepsis is difficult because of the variable and nonspecific clinical presentation of this condition. It is extremely important to make an early diagnosis of sepsis, because prompt institution of antimicrobial therapy improves the outcomes [9].

The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby. Confirmation of the diagnosis may take time, and diagnostic tests are used to obtain a rapid indication of the infection status [15].

IL-6 belongs to the family of cytokines. It is one of the mediators of inflammation that are released early in the course of septic shock and is crucial in initiating the immune response, as well as the activation of T lymphocytes and B lymphocytes and lymphocyte proliferation and differentiation. In addition, IL-6 is a potent pyrogen. It also induces the release of acute-phase proteins like CRP [8].

IL-6 reaches its peak after 2 h of bacterial stimulus onset; thus, its level may be elevated before the start of the symptoms and before the rise of routinely used markers [9]. IL-6 was reported to appear earlier in plasma than CRP [10].

The purpose of this case–control study was to evaluate the role of cord blood IL-6 as a predictor of early-onset neonatal sepsis in the preterm group.

This cross-sectional case–control study was carried out at Benha University Hospitals. It included 75 neonates with a suspicion of sepsis. Patients were classified into three groups according to their gestational age and subdivided according to sepsis into the proven sepsis group (12 cases), the probable sepsis group (48 cases) and the noninfected group (15 cases). Samples were taken from the patients early just upon birth.

The mean body weight in the proven sepsis group was 1.16 kg, that in the probable sepsis group was 1.5 kg and that in the noninfected group was 1.97 kg.

Our study showed that the mean body weight was significantly larger in the noninfected group than in the septic groups ($P < 0.001$). This agrees with the study of Schrag *et al.* [16] who found that factors associated with EOS included preterm delivery [adjusted relative risk (aRR) = 2.6; 95% confidence interval (CI): 1.4–4.8], low birth weight (<1500 g: aRR = 6.5; 95% CI: 2.4–17.3), meconium-stained amniotic fluid (aRR = 2.8; 95% CI: 2.2–3.7) and first birth (aRR = 1.8; 95% CI: 1.4–2.3).

In the current study, normal vaginal delivery and caesarean section showed no association with increased sepsis risk. This agrees with the study by Alfirevic *et al.* [17], who stated that there was also no difference between the caesarean or vaginal delivery groups in terms of markers of possible birth asphyxia [relative risk (RR) 1.63; 95% CI: 0.84–3.14; one trial, 12 women] or Apgar score less than 7 at 5 min (RR 0.83; 95% CI: 0.43–1.60; four trials, 115 women) and no difference in attempts at breast-feeding (RR 1.40; 95% CI: 0.11–17.45; one trial, 12 women). There was also no difference in neonatal fitting/seizures (RR 0.22; 95% CI: 0.01–4.32; three trials, 77 women), hypoxic ischaemic encephalopathy (RR 4.00; 95% CI: 0.20–82.01; one trial, 12 women) or respiratory distress syndrome (RR 0.55; 95% CI: 0.27–1.10; three trials, 103 women). There were no data reported in the trials specifically relating to meconium aspiration. There was also no significant difference between the two groups with respect to abnormal follow-up in childhood (RR 0.65; 95% CI: 0.19–2.22; one trial, 38 women) or delivery less than 7 days after entry (RR 0.95; 95% CI: 0.73–1.24; two trials, 51 women).

However, Bourgeois-Nicolaos *et al.* [18], Namavar *et al.* [19] and Stoll *et al.* [20] found that normal vaginal delivery was associated with higher risk for sepsis. In contrast, Malloy [21] suggests that primary caesarean section may pose an increased risk for neonatal mortality and morbidity in low-risk preterm infants at 32–36 weeks' gestation, independent of any reported risk factors.

These different results may be attributed to good sterilization and intrapartum chemoprophylaxis, which markedly decrease the risk for sepsis in neonates delivered by vaginal delivery.

In the current study, it was found that the septic groups had highly significant increase in the occurrence of PROM greater than 18 h, maternal fever and chorioamnionitis when compared with the noninfected group.

These results agree with the study by Ananthakrishnan and Gunasekaran [22], who stated that PROM greater than 18 h, maternal urinary tract infection and chorioamnionitis are risk factors for early-onset neonatal sepsis. Also, similar results were reported by Shah *et al.* [23], Kaufman and Fairchild [24] and Ottolini *et al.* [25].

Cernada *et al.* [11] reported that WBC count did not differ significantly between septic and nonseptic very-low-birth-weight babies. In contrast, I/T ratio and total leucocytic count (TLC) showed significant increase in the septic groups compared with the nonseptic group among preterm babies ($P < 0.001$)). Terrin and colleagues reported that I/T ratio was higher in septic patients than in nonseptic or control babies.

In our study, IL-6 was significantly higher in the septic groups (probable and proven) with median 371.92 and 124.85 pg/ml, respectively, than in the noninfected group, with median 8.53 pg/ml ($P < 0.0001$).

Similar results were found by Beceiro Mosquera *et al.* [26], who conducted a study on 42 cases and classified them into confirmed or probable infection groups and found that serum IL-6 was high in both groups, and there was no significant difference regarding serum IL-6 between the two.

Further, Abdollahi *et al.* [27] stated that IL-6 was higher in patients with clinical evidence of sepsis and even higher in those with a positive blood culture. Similar findings were reported by Khassawneh *et al.*[28] and Dollner *et al.* [29].

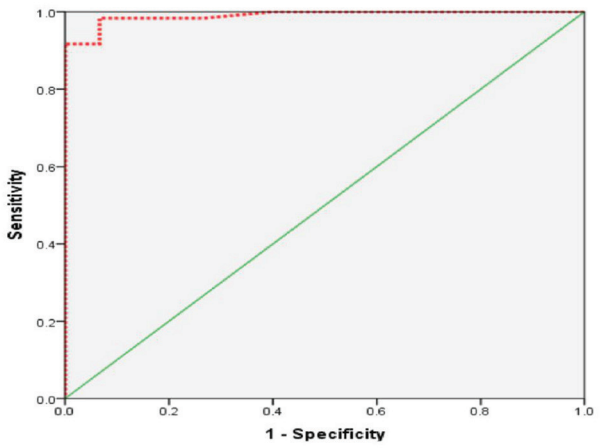
We found that IL-6 was significantly higher in the septic preterm infants compared with the nonseptic ones; thus, immature infants seem to be capable of synthesizing this cytokine, which agrees with the study of Dollner *et al.* [30] and Messer *et al.* [31].

We found higher levels of serum IL-6 with more severe illness and longer duration of stay. This agrees with the results of Harris *et al.* [32], who found that patients with higher levels of serum IL-6 had more severe illness and worse outcome. Our study results disagree with those of Messer *et al.* [31], who found no correlation between serum IL-6 and duration of admission. Moreover Ng *et al.* [33] found that sepsis patients who developed disseminated intravascular coagulopathy had high IL-6 levels.

Buck *et al.* [34] observed that IL-6 level decreases to normal even if the infection continues and reported falling levels of IL-6 even in patients with persistent symptoms of endotoxic shock. The half-life of IL-6 is short for different reasons such as binding to plasma proteins such as α_2-macroglobulin, early storage in the liver, or inhibition by other cytokines. This leads to false-negative findings when sampling is performed later in the course of the disease, which could not be confirmed by our study as it needs follow-up determination of IL-6 for each patient.

In our study, on the basis of sensitivity and specificity of the results, cord blood IL-6 at cutoff point of 47.5 pg/ml or more can be used as a marker for the diagnosis of neonatal sepsis in neonates with suspicion of infection with sensitivity 98.3% and specificity 93.3%.

Figure 1



Receiver operating characteristic (ROC) curve for cord blood interleukin-6 (IL-6).

A systematic review with meta-analysis was conducted by Shahkar *et al.* [35]. Meta-analysis was performed on 13 publications including 353 infants with sepsis and 691 control infants. The pooled sensitivity and specificity of IL-6 were 0.79 and 0.84, respectively. The maximum joint sensitivity and specificity (i.e. the *Q* value) in the summary receiver operating characteristic (SROC) curve was 0.82 and the area under the curve was 0.89 (95% CI: 0.84–0.94) (Fig. 1).

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Conflicts of interest
There are no conflicts of interest.

References

1 Paolucci M, Landini MP, Sambri V. How can the microbiologist help in diagnosing neonatal sepsis? Int J Pediatr 2012; **2012**:120–139.

2 Wu JH, Chen CY, Tsao PN, Hsieh WS Chou HC. Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. Pediatr Neonatol 2009; **50**:88–95.

3 Weston EJ, Pondo T, Lewis MM, Martell-Cleary P, Morin C, Jewell B, *et al.* The burden of invasive early-onset neonatal sepsis in the United States, 2005–2008., Pediatr Infect Dis J 2011; **30**:937–941.

4 Tissières P, Ochoda A, Dunn-Siegrist I, Drifte G, Morales M, Pfister R, *et al.* Innate immune deficiency of extremely premature neonates can be reversed by interferon-γ. PLoS One 2012; **7**:e32863.

5 Bhatti M, Chu A, Hageman J, *et al.* Future directions in the evaluation and management of neonatal sepsis. Neoreviews 2012; **13**:e103.

6 Terrin G, Passariello A, Manguso F, Salvia G, Rapacciuolo L, Messina F, *et al.* Serum calprotectin: an antimicrobial peptide as a new marker for the diagnosis of sepsis in very low birth weight newborns. Clin Dev Immunol 2011; **2011**:291085.

7 Kayange N, Mwizamholya D, Kamugisha E, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital. Pediatrics 2010; **39**:1471–2431.

8 Tamayo E, Fernández A, Almansa R, Carrasco E, Heredia M, Lajo C, *et al.* Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw 2011; **22**: 82–87.

9 Campos DP, Silva MV, Machado JR, Castellano LR, Rodrigues V, Barata CH. Early-onset neonatal sepsis: cord blood cytokine levels at diagnosis and during treatment. J Pediatr (Rio J) 2010; **86**:509-514.

10 Clendenen TV, Koenig KL, Arslan AA, Lukanova A, Berrino F, Gu Y, *et al.* Factors associated with inflammation markers, a cross-sectional analysis. Cytokine 2011; **56**:769–778.

11 Cernada M, Badía N, Modesto V, Alonso R, Mejías A, Golombek S, Vento M. Cord blood interleukin-6 as a predictor of early-onset neonatal sepsis. Acta Paediatr 2012; **101**:e203–e207.

12 Lam HS, Ng PC. Biochemical markers of neonatal sepsis. Pathology 2008; **40**:141–148.

13 Kurt AN, Aygun AD, Godekmerdan A, Kurt A, Dogan Y, Yilmaz E. Serum IL-1beta, IL-6, IL-8, and TNF-alpha levels in early diagnosis and management of neonatal sepsis. Mediators Inflamm 2007; **2007**:31397.

14 Stoll BJ, Hansen NI, Sánchez PJ, Faix RG, Poindexter BB, Van Meurs KP, *et al.*, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatrics 2011; **127**:817–826.

15 Meem M, Modak JK, Mortuza R, Morshed M, Islam MS, Saha SK. Biomarkers for diagnosis of neonatal infections: a systematic analysis of their potential as a point-of-care diagnostics. J Glob Health 2011; **1**: 201–209.

16 Schrag SJ, Cutland CL, Zell ER, Kuwanda L, Buchmann EJ, Velaphi SC, *et al.* Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of perinatal sepsis trial, Soweto, South Africa. Pediatr Infect Dis J 2012; **31**:821–826.

17 Alfirevic Z, Milan SJ, Livio S. Caesarean section versus vaginal delivery for preterm birth in singletons. Cochrane Database Syst Rev 2012; **6**:CD000078.

18 Bourgeois-Nicolaos N, Lucet JC, Daubié C, Benchaba F, Rajguru M, Ruimy R, *et al.* Maternal vaginal colonisation by Staphylococcus aureus and newborn acquisition at delivery. Paediatr Perinat Epidemiol 2010; **24**:488–491.

19 Namavar Jahromi B, Poorarian S, Poorbarfehee S. The prevalence and adverse effects of group B streptococcal colonization during pregnancy. Arch Iran Med 2008; **11**:654–657.

20 Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, *et al.* Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 1996; **129**:72–80.

21 Malloy MH. Impact of cesarean section on intermediate and late preterm births: United States, 2000-2003. 2009;**36**:26-33. doi: 10.1111/j.1523-536X.2008.00292.x.

22 Ananthakrishnan S, Gunasekaran D. Etiology and risk factors for early onset neonatal sepsis. Indian J Med Microbiol 2009; **27**:279.

23 Shah GS, Budhathoki S, Das BK, Mandal RN. Risk factors in early neonatal sepsis. Kathmandu Univ Med J 2006 **4**:187–191.

24 Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004; **17**: 638–680.

25 Ottolini M, Lungren K, Cason S. Utility of complete blood count and blood culture screening in the diagnosis of neonatal sepsis. Pediatr infect Dis J 2003; **17**:222–235.

26 Beceiro Mosquera J, Sivera Monzo CL, Oria de Rueda Salguero O, Olivas López de Soria C, Herbozo Nory C. Usefulness of a rapid serum interleukin-6 test combined with C-reactive protein to predict sepsis in newborns with suspicion of infection. An Pediatr (Barc) 2009; **71**:483–488.

27 Abdollahi A, Shoar S, Nayyeri F, Shariat M. Diagnostic value of simultaneous measurement of procalcitonin, interleukin-6 and hs-CRP in prediction of early-onset neonatal sepsis. Mediterr J Hematol Infect Dis 2012; **4**:e2012028.

28 Khassawneh M, Hayajneh WA, Kofahi H, Khader Y, Amarin Z, Daoud A. Diagnostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6 and immunoglobulin M. Scand J Immunol 2007; **65**:171–175.

29 Dollner H, Vatten L, Austgulen R. Early diagnostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6, soluble tumour necrosis factor receptors and soluble adhesion molecules. J Clin Epidemiol 2001; **54**:1251–1257.

30 Doellner H, Arntzen KJ, Haereid PE, Aag S, Austgulen R. Interleukin-6 concentrations in neonates evaluated for sepsis. J Pediatr 1998; **132**: 295–299.

31 Messer J, Eyer D, Donato L, Gallati H, Matis J, Simeoni U. Evaluation

of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996; **129**:574–580.

32 Harris MC, Costarino AT Jr, Sullivan JS, Dulkerian S, McCawley L, Corcoran L, *et al.* Cytokine elevations in critically ill infants with sepsis and necrotizing enterocolitis. J Pediatr 1994; **124**:105–111.

33 Ng PC, Li K, Leung TF, Wong RP, Li G, Chui KM, *et al.* Early prediction of sepsis-induced disseminated intravascular coagulation with interleukin-10, interleukin-6, and RANTES in preterm infants. Clin Chem 2006; **52**:1181–1189.

34 Buck C, Bundschu J, Gallati H, Bartmann P, Pohlandt F. Interleukin-6: a sensitive parameter for the early diagnosis of neonatal bacterial infection. Pediatrics 1994; **93**:54–58.

35 Shahkar L, Keshtkar A, Mirfazeli A, Ahani A, Roshandel G. The role of IL-6 for predicting neonatal sepsis: a systematic review and meta-analysis. Iran J Pediatr 2011; **21**:411–417.