Cord blood cytokine levels in focal early-onset neonatal infection after preterm premature rupture of membranes

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This study aimed to evaluate the levels of pro- and anti-inflammatory cytokines in umbilical cord blood of preterm neonates who developed focal early-onset infection (EOI) after preterm premature rupture of membranes (PPROM). This is a prospective study conducted on 46 preterm infants from mothers with PPROM. The cytokines were measure by flow cytometry. Newborns were classified into two groups as focal EOI (n=19) and non-infected (n=27). Interleukin (IL)-6 and IL-8 levels were higher, whereas IL-10 and IL-12 p70 levels were lower in the EOI when compared to the non-infected group. The best combination of cytokines was IL-6+IL-8, with a diagnostic accuracy of 0.97. Focal EOI after PPROM is associated with increased levels of IL-6 and IL-8 and diminished IL-10 and IL-12 in the cord blood of preterm infants. Combined assessment of IL-6 and IL-8 in cord blood may provide an additional tool for identifying preterm infants who develop EOI after PPROM.

Key words: early infection, in vivo inflammation, cytokine, innate immunity, infant, premature.

Preterm premature rupture of membranes (PPROM) is an adverse obstetric event found in one-third of preterm births, and it is a risk factor for early-onset infection (EOI)[1-5]. Sepsis and pneumonia are the main manifestations of infection in the first 28 days of life and carry high risks of morbidity and mortality, accounting for 750,000 to 1.2 million annual neonatal deaths in the world[6,7]. Despite the beneficial effects of intrapartum chemoprophylaxis in PPROM preventing group B Streptococcus (GBS) infection, there was no reduction in the rates of EOI caused by other microorganisms[8].

In a recent multicenter clinical trial on EOIs of very-low-birth-weight infants, the incidence of early-onset pneumonia was 8.6% and of early-onset sepsis (EOS) was 7%. Overall, PROM was observed in 25% of deliveries, increasing to 32% among infected neonates. Antibiotic prophylaxis was applied during 42% of the deliveries, and had no beneficial effect on the incidence of EOI[9].

Diagnosis of neonatal infection is always a challenge for neonatologists due to the non-specific clinical manifestations and poor accuracy of the laboratory tests. A delayed start of antibiotic treatment may lead to a fulminant course of sepsis, with death or major sequelae[6,10,11].

Some pro- and anti-inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1-beta, IL-6, IL-8, IL-10, and IL-12 p70 have been proposed as biomarkers for several neonatal diseases associated with fetal inflammatory response syndrome (FIRS), including neonatal infection, necrotizing enterocolitis, intraventricular hemorrhage, cerebral palsy, and chronic lung disease[12-16].

To date, the simultaneous measurement of several cytokines for combined analysis has

not been very common in neonatal research, since the blood volume needed to quantify cytokines by conventional enzyme-linked immunosorbent assay (ELISA) method is large, a requirement that limits its use in neonatal studies. However, the use of these biomarkers is very promising, because modern technologies allow simultaneous rapid measurement of several inflammatory mediators using a small volume of biological material. Thereby, the profile characterization of multiple cytokines in a single sample using multiplex technology by flow cytometry opens new perspectives into neonatal research, and its use may be an auxiliary tool in the diagnosis of neonatal infection and in the withdrawal of antibiotic therapy in the newborn at a high risk for infection[17].

Several studies have investigated the levels of inflammatory cytokines in the amniotic fluid and plasma of women with PPROM, in an attempt to associate them with maternal or neonatal infection, adding more knowledge to the pathophysiology of PPROM. Similar attempts have been conducted in early-onset neonatal infection studies using serum, plasma, umbilical cord blood, and urine from neonates born after PPROM[18-21]. However, the main focus in these studies has been neonatal sepsis associated with an intense systemic inflammatory response and not a focal infection with mild systemic inflammation. The purpose of this study was to evaluate levels of IL-1-beta, IL-6, IL-8, IL-10, IL-12 p70, and TNF-alpha in cord blood of newborns with focal EOI after PPROM.

Material and Methods

This was a prospective study including premature newborns of mothers with PPROM, born at the University Hospital maternity unit of Botucatu Medical School, UNESP, during the period 2007-2008. The study was approved by the local Research Ethics Committee, and written informed consent was obtained from a parent of each enrolled infant.

Inclusion criteria were: liveborn infants from singleton pregnancies, with amniotic membrane rupture time >12 hours, and gestational age <37 weeks. Gestational age was determined by the best obstetric estimate from the last menstrual period or ultrasound in the first trimester of pregnancy. Focal early infection was defined as: clinical signs of infection within 48 hours after birth, associated with abnormal biochemical and hematological parameters, and a primary focus identified. The clinical signs and symptoms included: lethargy, temperature instability, apnea, respiratory distress, bradycardia, feeding intolerance, and glucose intolerance[21]. Pneumonia, the main focus of early-onset neonatal infection, was diagnosed according to the following criteria[9, 22]:

- need of oxygen therapy, associated with suggestive chest radiography signs (new or progressive infiltrates) and

- at least one of the following clinical signs: thermal instability (axillary temperature >37.5°C or <36.0°C), increase in respiratory secretion, apnea, tachypnea, signs of respiratory distress, and

- at least one laboratory abnormality: C-reactive protein >1.0 mg/dl, hematologic Rodwell score ≥3.

Infants with chronic congenital infection, major congenital anomalies or who died in the first 24 hours after birth, without sufficient time to identify or rule out infection, were excluded.

All preterm infants delivered from PPROM were evaluated within the first 12 hours after birth to confirm or rule out infection. The investigation included: complete blood cell count, differential white cell and platelet counts, serial C-reactive protein evaluation, cerebrospinal fluid analysis, chest X-ray, and blood culture. Infants were classified into two groups as infected and non-infected.

Gestational and neonatal data analyzed were: maternal urinary and vaginal infection, antenatal corticosteroid, intrapartum antibiotic therapy, cesarean section, clinical and histological chorioamnionitis, fetal distress, gender, gestational age, birth weight, Apgar score, and the levels of pro- and anti-inflammatory cytokines in umbilical cord blood.

Cytokine Measurement

A 2 ml blood sample was collected from the umbilical vein immediately after birth, centrifuged for 10 minutes (min) at 1500 *g*, and stored at -80°C in a freezer until cytokine determination.

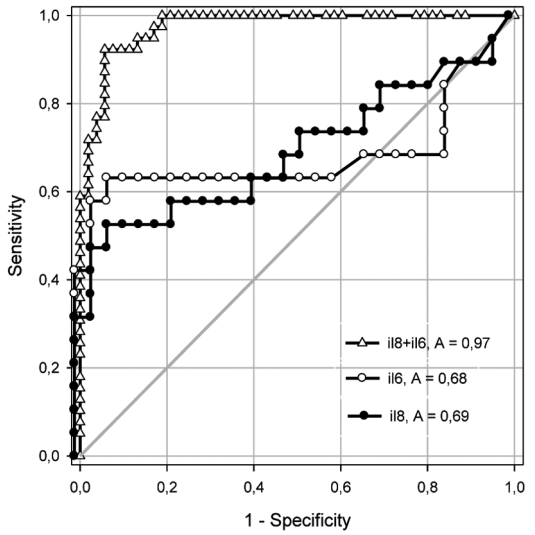

Fig. 1. Receiver operating characteristic (ROC) curve analysis for IL-6, IL-8 and combined analysis of IL-6 + IL-8 levels in cord blood plasma of the EOI (early-onset infection) group. The areas under the ROC curve (A) for IL-6, IL-8 and their combination were 0.68, 0.69 and 0.97, respectively.

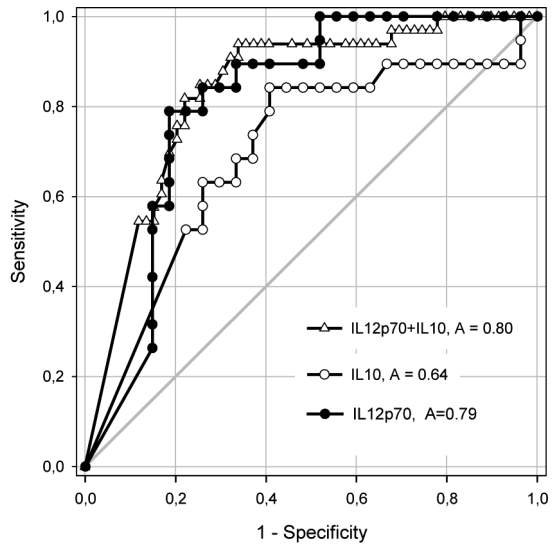

Fig. 2. Receiver operating characteristic (ROC) curve analysis for IL-10, IL-12 p70 and combined analysis of IL-10 + IL-12p70 levels in cord blood plasma of the EOI (early-onset infection) group. The areas under the ROC curve (A) for IL-10, IL-12 p70 and their combination were 0.64, 0.79 and 0.80, respectively.

Interleukin (IL)-1-beta, IL-6, IL-8, IL-10, IL-12 p70, and TNF-alpha concentrations were measured using a FACSCalibur Flux Cytometer and Human Inflammation kit, (Becton Dickinson Biosciences, San Jose, CA, USA). Immunoassays were designed for a microsphere sensitized with a specific monoclonal antibody for each cytokine (Becton Dickinson Biosciences, San Jose, CA, USA). Minimum detection limits were 7.2 pg/ml for IL-1-beta, 2.5 pg/ml for IL-6, 3.6 pg/ml for IL-8, 3.3 pg/ml for IL-10, 1.9 pg/ml for IL-12 p70, and 2.5 pg/ml for TNF-alpha. The inter-assay variability was 15% and intra-assay variability was < 10% in all analyses.

Statistical Analysis

Comparison between the EOI and non-infected groups regarding the clinical variables was made through a test for differences of proportions (chi-square). Data from newborns and cytokines were expressed as mean and standard deviation when symmetric or as median and quartiles when asymmetric. Mann-Whitney or *t* test was used for comparing these variables between the EOI and non-infected groups. The *P* value adopted was ≤0.05. The accuracy of cytokines in distinguishing infected from non-infected neonates was analyzed by the Receiver Operating Characteristics (ROC) curve. The cutoff values for each cytokine were obtained from the inflection point on the ROC curve. These cutoff values enabled us to calculate the diagnostic performance of the cytokines individually and in combination. Data were presented with a 95% confidence interval (95% CI). All analyses were made using the Statistical Package for the Social Sciences (SPSS) software, 5.19.0.

Results

A total of 49 preterm infants born after PPROM satisfied the inclusion criteria; of these, one was excluded due to chronic congenital infection. Blood culture was obtained in all of the remaining 48 neonates; however, all cultures were negative and only two neonates had suspected EOS, but they were excluded because the diagnosis could not be reliably confirmed. Nineteen neonates had focal EOI, all of them characterized as pneumonia, and 27 were non-infected. Gestational and neonatal data are summarized in Tables I and II, respectively. Maternal urinary tract infection, antenatal corticosteroid use, and histological chorioamnionitis were more frequent in the EOI group (Table I). Neonates with EOI had a lower gestational age (Table II).

Table I. Gestational Data (Percentage) in the Groups: Early-Onset Infection (EOI) and Non-Infected

	EOI (n=19)	Non-infected (n=27)	p value*
Urinary infection	63%	22%	0.013
Vaginal infection	32%	22%	0.513
Antenatal steroids	79%	44%	0.042
Intrapartum antibiotics	56%	70%	0.608
Clinical chorioamnionitis	32%	7%	0.051
Histological chorioamnionitis	59%	8%	0.001
Fetal distress	28%	24%	1.000
Cesarean section	37%	33%	0.952

* chi-square test

The median cord blood IL-1-beta and TNF-alpha levels were similar in both groups (Table II). However, preterm neonates with early-onset pneumonia had significantly higher IL-6 and IL-8 levels than non-infected infants (Table II).

In contrast, IL-10 and IL-12 p70 concentrations were significantly lower in infected compared to non-infected preterm infants (Table II), and the median value of the IL-12 p70/IL-10 ratio in the EOI group was 0.030 (P25 = 0.01 - P75 = 0.31), significantly lower than the 3.33 in the non-infected group (P25 = 2.48 - P75 = 3.74) ($p<0.001$).

The diagnostic accuracies of IL-6, IL-8, IL-10, and IL-12 individually and in combination are shown in Table III.

Figure 1 shows the ROC curves for Il-6 and Il-8 individually and in combination. The combination of IL-6 and Il-8 presented the largest area under the ROC curves. The ROC curves for IL-10 and IL-12 are displayed in Figure 2. Combining these cytokines did not improve the diagnostic accuracy.

Discussion

Our clinical findings revealed higher incidences of maternal urinary tract infection and chorioamnionitis, as well as lower gestational age, in the EOI group. Intrauterine infection/inflammation and genitourinary tract infection have been associated frequently with preterm labor and preterm delivery[23,24]. A similar association has been described with

Table II. Neonatal Data by Group: Early-Onset Infection (EOI) and Non-Infected

	EOI (n=19)	Non-infected (n=27)	p value
Gestational age (weeks), mean ± SD*	31 ± 2	33 ± 1	0.013
Birth weight (g), mean ± SD*	1894 ± 556	2100 ± 330	0.228
Male gender, %**	68%	94%	0.746
Apgar 1st min, median (p25, p75) #	8 (6, 8)	7 (7, 8)	0.461
AGA (%)	84%	85%	1.000
IL-1 beta, median (p25, p75)#	2 (0, 4)	5.3 (0, 15)	0.071
IL-6, median (p25, p75)#	244 (3, 916)	7 (5, 21)	0.046
IL-8, median (p25, p75)#	135 (14, 325)	21 (10, 46)	0.034
IL-10, median (p25, p75)#	0 (0, 2.9)	4 (1, 6)	0.023
IL-12 p70, median (p25, p75)#	2 (0, 4)	9 (5, 20)	0.01
TNF-alpha, median (p25, p75)#	0.1 (0, 0.1)	4 (0, 13)	0.789

AGA: Appropriate-for-gestational age. IL: Interleukin. SD: Standard deviation. TNF: Tumor necrosis factor. *Student's t test; **Chi-square test; # Mann-Whitney U test.

Table III. Descriptive Data Obtained from the Analysis of ROC Curves for Cytokines

	IL-6	IL-8	IL-6 + IL-8	IL-10	IL-12 p70	IL-10 + IL-12
Cutoff	16.0	26.6	--	2.4	4.6	--
Sensitivity %	63.2	57.9	92.3	63.2	78.9	75.8
Specificity %	66.7	59.3	94.3	66.7	81.5	78.0
AUC	0.68	0.69	0.97	0.64	0.79	0.80
95% CI-AUC	0.48-0.86	0.51-0.86	0.95-0.99	0.54-0.86	0.68 - 0.93	0.75-0.92
PPV%	66.6	52.4	68.4	57.1	75.0	64.7
NPV%	80.7	68.0	81.5	73.1	84.6	71.4

AUC: Area under the curve. CI: Confidence interval. NPV: Negative predictive value. PPV: Positive predictive value.

chorioamnionitis, an important risk factor for early-onset neonatal infection and increased acute neonatal morbidity[9,25]. These findings highlight the relationship between histological chorioamnionitis and early-onset neonatal infection.

In recent years, some studies have demonstrated a relationship between pro- and anti-inflammatory cytokines and newborn infection[27-29], but few studies have evaluated their levels in the cord blood of preterm infants with focal EOI in the context of PPROM. The levels of cytokines have usually been investigated as markers of EOS in infants born after PPROM and also exposed to other obstetric pathologies, which could influence the results. Our study evaluated only preterm neonates with early-onset pneumonia after PPROM, without exposition to other obstetric diseases, in order to clarify the role of pro- and anti-inflammatory cytokines in EOI.

The main result of our study was the finding of increased levels of IL-6 and IL-8 in umbilical cord blood from preterm infants who developed early-onset pneumonia after PPROM, suggesting that these cytokines can be reliable and useful biomarkers not only for severe neonatal infections like sepsis, but also in focal EOI[30]. In contrast, other inflammatory cytokines such as IL-1-beta and TNF-alpha did not differ between the groups.

The results of IL-6 and IL-8 in our preterm infants with early-onset pneumonia were very similar to those reported by Dollner et al.[31] in cord blood from neonates who developed EOS. Although there are some methodological differences, such as the fact that the Dollner study enrolled preterm and term neonates with sepsis, of whom only 29% had been exposed to PPROM, these results confirm our hypothesis that these cytokines could be helpful biomarkers for focal EOI, and they reinforce the need for additional research on focal infections.

A previous *in vitro* study demonstrated that the production of IL-1-beta, IL-6 and IL-8, when induced by agonists of several Toll-like receptors (TLR) found in mononuclear cells of cord blood, is higher than production by adult mononuclear cells, thus suggesting the potential role of these cytokines as biomarkers in newborn diseases and refusing the possibility of low production due to a limited innate immune response. However, our results did not show increased cord blood levels of IL-1-beta and TNF-alpha in preterm infants with focal EOI[20, 21, 32]. This may be attributable to the type of TLR stimulation responsible for the production of IL-1 and TNF-alpha or to the variability in the gestational age of the newborn infants evaluated[32]. Some receptors associated with the production of inflammatory cytokines present a low level of expression in infections, as in the case of TLR4[33]. Our results on TNF-alpha levels in the infected group are in agreement with studies that found defective TNF-alpha production by neonates[20, 21, 33].

Despite their great promise, none of these cytokines as an individual test reliably confirms or excludes infection in neonates at risk; therefore, their combined use has been proposed[10,16,20,33]. We have demonstrated that the diagnosis of early-onset pneumonia in preterm infants born after PPROM can be established or ruled out with a high level of confidence by simultaneous measuring of IL-6 and IL-8 levels from cord blood. The area under the ROC curve was 0.97, with high sensitivity

and specificity.

An interesting finding in this study was the lower levels of IL-10 and IL-12 p70 in neonates who developed infection, suggesting impairment in immune regulatory cytokines that drive Th2 cells, as has been demonstrated previously[32,34]. Additionally, our results showed a significantly lower IL-12/IL-10 ratio in infected preterm infants, which highlights their greater IL-12 deficiency. The area under the curve of IL-10 was lower than that of IL-12, while combined analysis did not increase the accuracy of these cytokines.

Data are very scarce on the role of IL-12 in neonatal infection. A recent study showed that preterm infants were deficient in IL-12 production, which may result in increased susceptibility to infection. Consistent with this assumption, IL-12 levels at birth were significantly lower in infants who developed EOS, whereas IL-6 was higher than in non-infected infants, indicating that low IL-12 was not a consequence of a blunted global inflammatory response to infection[35]. The stimulation of IL-12 p70 production by TLR in the cord blood of healthy term infants was diminished compared to that in adult blood. This abnormal TLR responsiveness with low IL-12 p70 has been associated with increased susceptibility to bacterial infections[32].

Data on anti-inflammatory IL-10 are somewhat controversial. Preliminary results suggest an immunosuppressive role for IL-10 in abnormal pregnancies, whereas in normal pregnancies, IL-10 was undetectable in cord blood[36]. More recently, an imbalance between the pro- and anti-inflammatory responses has been proposed to explain the enhanced inflammatory response in infected neonates, and reduced IL-10 production after stimulation was observed in term and preterm neonates compared to adults, suggesting an immature anti-inflammatory response in newborn infants[37]. Similar IL-10 responses after *in vitro* stimulation have been reported in preterm compared to term neonates[35]. Increased IL-10 gene expression in cord blood mononuclear cells was detected in preterm infants exposed to funisitis, which might modulate the postnatal immune response[38]. Our results showed lower IL-10 levels in infected preterm infants, which supports the hypothesis of an imbalance between the pro- and anti-inflammatory responses in infected preterm infants.

We are aware that our results are based on a small number of neonates, which allows limited inference about the inflammatory mechanisms, and thus they need to be interpreted with caution. However, we measured multiple cytokines by the cytometric bead array technique and evaluated the cytokine profile in a specific neonatal disease - early-onset pneumonia in preterm infants born after PPROM - a common problem in clinical practice, which has been investigated only scarcely until now.

This study adds new information regarding the role of cytokines in the diagnosis of focal neonatal infections such as pneumonia. Since PPROM is an important cause of prematurity, and the main differential diagnosis of respiratory distress in this condition is the respiratory distress syndrome, an early diagnosis or exclusion of pneumonia may contribute to avoiding unnecessary antibiotic therapy.

In summary, focal EOI after PPROM is associated with increased IL-6 and IL-8 levels and decreased IL-10 and IL-12 levels in the cord blood of preterm infants. Our results confirm that cord blood IL-6 and IL-8 levels may be an additional tool for investigating EOI after PPROM, while the combined assessment of IL-6 and IL-8 should be included in diagnostic algorithms of EOI in neonates after PPROM. More studies addressing the role of IL-10 and IL-12 in EOI are needed.

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REFERENCES

1. Merenstein GB, Weisman LE. Premature rupture of the membranes: neonatal consequences. Semin Perinatol 1996; 20: 375-380.
2. Aagard-Tillery KM, Nuthalapaty FS, Ramsey PS, Ramin KD. Preterm premature rupture of membranes: perspectives surrounding controversies in management. Am J Perinatol 2005; 22: 287-297.

3. Menon R, Fortunato SJ. Infection and the role of inflammation in preterm premature rupture of the membranes. Best Pract Res Clin Obstet Gynaecol 2007; 21: 467-478.

4. Buchanan SL, Crowther CA, Levett KM, Middleton P, Morris J. Planned early birth versus expectant management for women with preterm prelabour rupture of membranes prior to 37 weeks' gestation for improving pregnancy outcome. Cochrane Database Syst Rev 2010; 3: CD004735.

5. Choudhury AM, Nargis S, Mollah AH, Kabir LM, Sarkar RN. Determination of risk factors of neonatal pneumonia. Mymensingh Med J 2010; 19: 323-329.

6. Nissen MD. Congenital and neonatal pneumonia. Pediatr Respir Rev 2007; 8: 195-203.

7. Duke T. Neonatal pneumonia in developing countries. Arch Dis Child Fetal Neonatal Ed 2005; 90: F211-219.

8. Stoll BJ, Hansen NI, Sánchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatrics 2011; 127: 817-826.

9. Wójkowska-Mach J, Borszewska-Kornacka M, Domañska J, et al. Early-onset infections of very-low-birth-weight infants in Polish neonatal intensive care units. Pediatr Infect Dis J 2012; 31: 691-695.

10. Franz AR, Bauer K, Schalk A, et al. Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. Pediatrics 2004; 114: 1-8.

11. Schrag SJ, Hadler JL, Arnold KE, et al. Risk factors for invasive, early-onset Escherichia coli infections in the era of widespread intrapartum antibiotic use. Pediatrics 2006; 118: 570-576.

12. Messer J, Eyer D, Donato L, et al. Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996; 129: 574-580.

13. Gomez R, Romero R, Ghezzi F, et al. The fetal inflammatory response syndrome. Am J Obstet Gynecol 1998; 179: 194-202.

14. Yoon BH, Romero R, Park JS, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol 2000; 182: 675-681.

15. Ng PC. Clinical trials for evaluating diagnostic markers of infection in neonates. Biol Neonate 2005; 87: 111-112.

16. Romero R, Erez O, Espinoza J. Intrauterine infection, preterm labor, and cytokines. J Soc Gynecol Investig 2005; 12: 463-465.

17. Labenne M, Lizard G, Ferdynus C, et al. A clinic-biological score for diagnosing early-onset neonatal infection in critically ill preterm infants. Pediatr Crit Care Med 2011; 12: 203-209.

18. Franz AR, Kron M, Pohlandt F, Steinbach G. Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. Pediatr Infect Dis J 1999; 18: 666-671.

19. Asrat T. Intra-amniotic infection in patients with preterm prelabor rupture of membranes: pathophysiology, detection, and management. Clin Perinatol 2001; 28: 735-752.

20. Berner R, Tüxen B, Clad A, Forster J, Brandis M. Elevated gene expression of interleukin-8 in cord blood is a sensitive marker for neonatal infection. Eur J Pediatr 2000; 159: 205-210.

21. Bentlin MR, Rugolo LM, Rugolo A Jr, Hashimoto M, Lyra JC. Is urine interleukin-8 level a reliable laboratory test for diagnosing late onset sepsis in premature infants? J Trop Pediatr 2007; 53: 403-408.

22. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 1998; 112: 761-767.

23. Romero R, Espinoza J, Chaiworapongsa T, Kalache K. Infection and prematurity and the role of preventive strategies. Semin Neonatol 2002; 7: 259-274.

24. Zanardo V, Vedovato S, Cosmi E, et al. Preterm premature rupture of membranes, chorioamnion inflammatory scores and neonatal respiratory outcome. BJOG 2010; 117: 94-98.

25. Hagberg H, Wennerholm UB, Sävman K. Sequelae of chorioamnionitis. Curr Opin Infect Dis 2002; 15: 301-306.

26. Ilievski V, Hirsch E. Synergy between viral and bacterial toll-like receptors leads to amplification of inflammatory responses and preterm labor in the mouse. Biol Reprod 2010; 83: 767–773.

27. Ng PC, Li K, Wong RP, et al. Pro-inflammatory and anti-inflammatory cytokine response in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed 2003; 88: F209-213.

28. Celik IH, Demirel FG, Uras N, et al. What are the cut-off levels for IL-6 and CRP in neonatal sepsis? J Clin Lab Anal 2010; 24: 407-412.

29. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol 2010; 37: 421-438.

30. Hatzidaki E, Gourgiotis D, Manoura A, et al. Interleukin-6 in preterm premature rupture of membranes as an indicator of neonatal outcome. Acta Obstet Gynecol Scand 2005; 84: 632-638.

31. Dollner H, Vatten L, Linnebo I, et al. Inflammatory mediators in umbilical plasma from neonates who develop early-onset sepsis. Biol Neonate 2001; 80: 41-47.

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

33. Caron JE, LaPine TR, Augustine NH, Martins TB, Hill HR. Multiplex analysis of toll-like receptor-stimulated neonatal cytokine response. Neonatology 2010; 97: 266-273.

34. Schelonka RL, Maheshwari A, Carlo WA, et al. T cell cytokines and the risk of blood stream infection in extremely low birth weight infants. Cytokine 2011; 53: 249-255.

35. Lavoie PM, Huang Q, Jolette E, et al. Profound lack of interleukin (IL)-12/IL-23p40 in neonates born early in gestation is associated with an increased risk of sepsis. J Infect Dis 2010; 202: 1754-1763.

36. Hata T, Kawamura T, Fujiwaki R, et al. Interleukin-4, interleukin-10, and soluble tumor necrosis factor receptors in cord blood. Gynecol Obstet Invest 1997; 43: 155-157.

37. Schultz C, Temming P, Bucsky P, et al. Immature anti-inflammatory response in neonates. Clin Exp Immunol 2004; 135: 130-136.

38. Wirbelauer J, Seidenspinner S, Thomas W, Kunzmann S, Speer CP. Funisitis is associated with increased interleukin-10 gene expression in cord blood mononuclear cells in preterm infants ≤32 weeks of gestation. Eur J Obstet Gynecol Reprod Biol 2011; 155: 31-35.