Immediate Diagnosis of Early Onset Sepsis in Premature Newborns by Measurement of Cord C-Reactive Protein and Interleukin-6

M.M. Gharehbaghi, G.A. Maamuri[1],
A. Peirovifar[2], H. Boskabadi[1],
J.T. Afshari[3], M.T. Shakeri[4]

Abstract

Background: The purpose of this study was to determine the relationship between early onset sepsis and increased levels of C-reactive protein (CRP) and interleukin-6 (IL-6) in cord plasma.

Methods: A prospective study was conducted in 141 premature infants delivered with gestational ages of 26-35 weeks. IL-6 and CPR were measured by enzyme-linked immunoassay in the cord plasma of the neonates. According to clinical, laboratory findings and blood culture results, newborn infants were allocated into four groups (A-D): documented early onset infection, clinical sepsis, possible infection, and control groups respectively.

Results: Mean IL-6 levels in group A-D was 264, 212, 160, and 33.3 pg/ml respectively. Difference between groups was statistically significant (p=.002). With cut off point of 18 pg/ml, the sensitivity and specificity of IL-6 for diagnosis of early onset sepsis was 72% and 55% respectively. There was not significant difference between mean levels of CRP among groups (p=0.28).

Conclusion: Having considered the relatively good sensitivity and moderate specificity of cord IL-6, using this test can be recommended as a useful detector of early onset sepsis and non-infected sick neonates.
Iran J Med Sci 2007; 32(4): 217-221.

Keywords ● Premature newborn ● sepsis ● CRP ● interleukin-6

Departments of pedatrics,
[2]Intensive Care Medicine,
Tabriz University of Medical Sciences,
Tabriz, Iran.
[1]Departments of pedatrics,
[3]Immunology, [4]Social Health
Mashad University of Medical Sciences,
Mashad, Iran.

Correspondence:
Manaizeh Mostafa Gharehbaghi MD,
Departments of pedatrics,
Tabriz University of Medical Sciences,
Tabriz, Iran.
Tel: +98 411 5262250-7
Fax: +98 411 5262280
Email: gharehbaghimm@yahoo.com

Introduction

Despite major advances in the management of premature newborn infants, both early and late onset infections remain important causes of neonatal morbidity and mortality.[1-3] Early diagnosis of neonatal bacterial infection is one of the greatest challenges to neonatologists. At the birth time, the diagnosis relies on the history of pregnancy and a number of well-defined risk factors.[4] Early clinical features of infection are often subtle, non-specific, and difficult to recognize, particularly in premature infants.[5-7]

Numerous parameters have been studied to establish early diagnosis of serious and critical bacterial infection in neonates. Unfortunately markers such as white blood cell count and immature to total neutrophil ratio (I: T) do not have good sensitivity and specificity in early diagnosis of neonatal sepsis.[4,8;9]

Regarding morbidity and mortality, clinical course and outcome of sepsis essentially depend on prompt diagnosis and immediate treatment with broad spectrum antibiotics. On the other hand, many newborns are unnecessarily treated with antibiotics without conclusive evidence of sepsis.

In recent years, proinflamatory cytokins have been proposed as markers for early diagnosis of infection. IL-6 is secreted from macrophages, fibroblasts, and endothelial cells and mediates some host defense responses to infection. After release of bacterial cell wall components, such as endotoxins, IL-6 level rises and reaches peak level in a few hours.[2,6-8] In chorioamnionitis, fetal infection, and funisitis there is elevated levels of IL–6 in cord blood.[10,11] This cytokine is the major inducer of hepatic synthesis of proteins such as CRP.[2,7,8,12]

IL–6 has a very short half life and its circulatory concentration can decrease after antimicrobial treatment and becomes undetectable within 24 hours. Its sensitivity will be changed during the course of sepsis. Its high sensitivity in early stages of infection (89% at 2 hr) decreases to 67% and 58% in 24 and 48 hours respectively.[1] The window opportunity of IL–6 for detecting infection is narrow. To improve its diagnostic capability in clinical practice, it should be used in conjunction with other markers such as CRP (as a late marker).[1,5] In early stages of neonatal sepsis, CRP has a good specificity and less sensitivity.[1,2,13-15]

In this study we compared the efficacy of umbilical cord plasma CRP and IL–6 as early indicator of neonatal sepsis in the first 72 hours of life, which is considered as early onset sepsis.

Patients and Methods

This observational study took place at Ghaem Hospital affiliated to Mashad University of Mediccal Sciences, Mashad, Iran. Over a period of 15 months (June 2005 to September 2006), 150 premature newborn infants with gestational ages of 26-35 weeks in the delivery room of this hospital were enrolled in this study. Informed written consent was obtained from the parents and the University's Regional Committee of Ethics in Medical Research approved this study.

Neonatal cord blood samples were obtained for determination of CRP and IL–6 levels immediately after delivery and cutting the cord. Excluded neonates were those with gestational ages over 35 weeks, meconial stained ones, and neonates with coexistent major malformations. Nine neonates were excluded from the study because of transfer to another hospital within 72 hours after birth and inadequate follow up (2 cases), sever hemolysis (3 cases), and inadequate blood sampling or missed labeling (4 cases).

Patients were evaluated for following seven clinical features:
1) Temperature instability including hypothermia, hyperthermia or >3 different recordings with changes more than 0.5ºC in 24 hours.
2) Respiratory problems including grunting, intercostals retraction, tachypnea, cyanosis, apnea more than 20 sec or > 15 sec associated with bradycardia, cyanosis or changes in skin color and muscular tone.
3) Cardiovascular problems including bradycardia more than 5 times in 24 hours or persisting tachycardia more than 170 beats / min.
4) Poor perfusion with prolonged capillary refilling (>3sec) or poor skin color.
5) Hypotension (systolic blood pressure less than 40 mmHg) requiring volume replacement or vasopressor infusion.
6) Neurological signs including irritability, seizure, and lethargy.
7) Gastrointestinal signs including distended abdomen, feeding intolerance, or hepatomegaly.

Abnormal laboratory findings in the first 72h included elevated I: T ratio more than 0.2, white blood cell count $\geq 25,000/mm^3$ or $\leq 5000/mm^3$, and platelet count $\leq 100 \times 10^9/lit$.

According to clinical or laboratory findings and results of blood cultures, the total 141 premature newborns were allocated to four different groups. Group A (early onset sepsis) consisted of 12 neonates who had clinical signs and symptoms of early onset infection documented by positive blood culture in the first 72 hours of life. In group B, 24 neonates were diagnosed as having clinical sepsis. They had at least three clinical and two laboratory findings indicative of sepsis but had negative blood culture. Group C consisted of 61 newborn infants with possible infection who had less than three clinical and one laboratory findings for sepsis. Their blood cultures were negative. And group D considered as a control group with 44 premature neonates without any clinical and laboratory features of infection in the first 72 hours. Except prematurity, there was no risk factor for infection in group D.

We considered maternal fever, prolonged rupture of membranes for ≥ 18 hours, uterine tenderness, fuel smelling and cloudy amniotic fluid as risk factors for neonatal infection. Clinical determination of infection was made by neonatologists who were blind to CRP and IL-6 levels in cord blood. Umbilical cord blood samples were obtained during delivery and centrifuged

immediately. Then the serum samples were stored in small aliquots at -70ºC until analysis. IL–6 and CRP concentrations were measured by enzyme–linked immunoassay (ELISA) with a detection limit of 1 pg/ml for IL–6 and 1 μg/ml for CRP. Human IL–6 ELISA (Bender med system, Vienna, Austria) was used for IL–6 measurement and CRP ELISA (IBL Hamburg Hamburg, Germany) was used for CRP measurement. Statistical analysis was performed using SPSS software version 11.5.

The inflammatory mediators had asymmetrical distribution and the difference between groups was analyzed by Kruskal–Wallis, one–way analysis of variance, and Mann Whitney U-test. Differences between symmetrically distributed variables were tested by one-way ANOVA and Student's *t* test, whereas differences between proportions (gender, gestational age) were tested by Chi square test. For all statistical analysis P<0.05 was considered significant. The receiver operating characteristics (ROC) method was used in order to establish the optimal cut–off point. Sensitivity, specificity, and positive and negative predictive values of selected cut-off levels were then calculated.

Results

Mean maternal age was 26.11±5.52 years. Mean gestational age of neonates was 31.86 ±2.29 weeks. And mean birth weight of them was 1410.70±310.04 grams. The maternal and neonatal characteristics of the study population are presented in table 1.

There were no significant differences between groups with respect to gender (p=0.57), mean Apgar score at 1 minute (p=0.36) and 5 minutes (p=0.39), maternal age (p=0.53), presence of pre-eclampsia during pregnancy (p= 0.31), and antepartum maternal antibiotic

treatment (p=0.89).

Table1: Maternal and neonatal characteristics.

	n	%
Maternal:		
Multiple gestation	28	25.7
Preeclampsia during pregnancy	25	22.9
Diabetics mellitus	5	4.6
Mode of delivery		
Vaginal	68	62
Cesarean section	41	38
Prolonged rupture of membranes	44	40.4
Clinical chorioamnionitis	9	8.3
Antepartum antibiotic administration	61	56
Total number	109	
Infant:		
Male gender	71	50
Low APGAR score	9	6
Respiratory distress	61	43
Exogenouse surfactant therapy	16	11
Total number	141	

The mean IL–6 values in cord blood was not significantly different between neonates in respect to maternal history of pre-eclampsia in pregnancy (P=0.317), but it was significantly higher in patients with history of PROM (p=0.000). The mean IL-6 levels in vaginally delivered infants were not significantly different from those delivered by Cesarean section (P=0.88).

The most common pathogens isolated in documented early onset sepsis were Klebsiella pneumonia and coagulase negative staphylococcus (CONS). E coli and staphylococcus areus were isolated in two cases.

Mean IL-6 levels in neonates who eventually expired were not significantly different from survivors (p=0.47). Mean CRP and IL–6 levels in umbilical cord blood among the groups are shown in table 2 and 3. Patients in group D had significantly lower IL6 levels than group A (P=0/018), B (P=0/007) and C (P=0/04)

Using a cut-off point of 18 pg/ml for IL–6, a sensitivity of 72% and specificity of 55% was

Table 2: Mean values of IL -6 (mg/ml) in cord blood of 4 patient groups.

Group	Number	Mean	SD	SE	95% confidence interval	
					Lower bound	Upper bound
A (documented EOS)	12	264.90	386.36	111.24	20.05	509.44
B (clinical sepsis)	24	232.12	274.59	56.05	116.17	348.08
C (infection possible)	61	160.0	269.85	34.55	90.90	229.12
D (control)	44	33.35	51.59	7.77	17.66	49.04

P value for IL-6 between groups was 0.002. EOS= Early Onset Sepsis, SD = Standard Deviation, SE = Standard Error

Table 3: Mean values of CRP (mg/ml) in the cord blood of patients in the four groups.

Group	No	Mean	SD	SE	95% confidence interval	
					Lower bound	Upper bound
A	12	4.67	10.39	3.00	-1.92	11.27
B	24	6.69	11.12	2.27	1.99	11.39
C	61	6.9	19.05	2.44	2.03	11.79
D	44	1.71	2.43	0.37	0.95	2.47

P value for CRP between groups was 0.28. A= Documented early onset sepsis, B=Clinical sepsis, C= Infection Possible, D= Control, SD=Standard Deviation, SE=Standard Error

obtained by ROC analysis. Negative predictive value (NPV) for detecting early onset sepsis was 92% and positive predictive value (PPV) was 33%. It means that normal IL-6 levels in cord blood sample are more likely to rule out sepsis in neonates.

Discussion

Determination of markers for diagnosis of early onset sepsis is evolving. Although white blood cell counts and ratio of immature to total neutrophil count (I: T ratio) have been used to diagnosis, they did not have good predictive value in diagnosis of early onset neonatal sepsis. Total white blood cell count exhibit a wide range of normality and machine measurement of neutrophil counts are inaccurate in the presence of nucleated red blood cells.[9] Assessment of neutrophil band form is subjective and requires an experienced hematologist to review the blood film.[4,8,9]

There is much interest for markers of infection that can reliably differentiate between infected and non-infected infants. In recent years proinflammatory cytokines have been proposed as markers for early diagnosis of sepsis.[13,14] In several studies IL–6 and CRP have been assessed for immediate diagnosis of sepsis.[1,13,16,17] In early onset sepsis combinations of tests have been evaluated in a few studies.[18-22] The reliability of CRP and IL-6 for differential diagnosis of infectious versus non-infectious diseases of newborns has been assessed in some studies and has yielded variable results due to variations in study design, wide range of gestational and postnatal age of study groups, different sample sizes, and different measurement methods.[8,12,22]

In our study similar to the previous ones,[8,12,23-25] there was no statistically significant difference for cord blood IL-6 levels between preterm infants with clinical sepsis and those with documented sepsis. This may reflect the high suggestive index of clinical signs and symptoms of sepsis. Most of infections in premature infants are acquired prior to birth. On the other hand, infection may be a major cause for prematurity.[4-7] In this study septic premature newborns and non-infected sick infants exhibited higher IL-6 concentrations than healthy premature newborns. It may be due to the hypothesis that IL-6 is an important mediator of host response to stress and infection and elevated cord IL-6 levels is an alarm for more investigations including blood culture, which remains gold standard for diagnosis of sepsis.

One of the most suspected mechanisms for preterm labor is premature activation of deciduas. Its activation may be due to occult upper genital tract infection. There is increasing evidence for relationship between preterm labor and upper genital tract infections.[2,4,5] In the study of Chiesa et al,[8,12] near-term healthy infants had significantly higher IL-6 concentrations than term healthy infants. So a gestational age dependent effect on the normal IL-6 values might be seen over the initial 48 hours of life. They concluded that higher IL-6 concentration in near-term healthy neonates might be the result of subclinical prenatal infection, which is common in preterm labor.

On the other hand, preterm delivery can be more stressful for fetus, which can induce increased cord IL-6 level. Other studies may be requiring for elucidating the influence of gestational age on cord IL-6 levels. We found 93% NPV for IL-6 that did not significantly differed from other studies.[22,24] Analysis of cord blood sample offers the advantages of determining cytokines at the earliest possible and well defined time.

Our study did not show CRP as an early marker of sepsis in cord blood. Elevated level of CRP is usually detectable after 6-8 hours of infection and its peak level is seen at 8-60 hours after the onset of the inflammatory process.[2,7] CRP has low positive predictive value,[1,18] and it may be more helpful if measured serially at 12, and 24 hours of life.[14,15,22]

Our study had some limitation. We evaluated CRP and IL-6 levels in one sample of cord plasma. It seems serial measurement of CRP may be more helpful for detection of sepsis.

Conclusion

With consideration of relatively good sensitivity and specificity of cord IL–6, it can be recommended using this test as a useful tool for early detection of neonatal sepsis and non-infective sick infants.

Acknowledgements

References

1   Behrman RE, Kliegman RM, Jenson HB. Nelson's Textbook of Pediatrics. 17th ed. Vol 1. Philadelphia; Saunders; 2004. p. 623-39.
2   Edwards MS. Postnatal bacterial infections. In: Martin RJ, Fanaroff AA, Walsh MC.

Neonatal–Perinatal Medicine: diseases of the fetus and infant. 8th ed. Vol 2 Philadelphia; Elsevier Mosby; 2006. p. 791-805.

3   Martius JA, Roos T, Gora B, et al. Risk factors associated with early onset sepsis in premature infants. *Eru J Obstet Gynecol Reprod Biol* 1999; 85: 151-8.

4   Janota J, Stranák Z, Bělohlávková S. Interleukin–6, Procalcitonin, C–reactive protein and immature to total neutrophil ratio (I/T) in the diagnosis of early onset sepsis in low birth weight neonates. *Ceska Gynekol* 2000; 1: 29-33.

5   Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; 340: 448-54.

6   Franz AR, Steinbach G, Kron M, Pohlandt F. Reduction of unnecessary antibiotic therapy in newborn infants using interleukin-8 and C-reactive protein as markers of bacterial infections. *Pediatrics* 1999; 104: 447-53.

7   Shelonka RL, Freij B J, Mc Crachen GH. Bacterial and fungal infections. In: Avery GB, Fletcher MA, MacDonald MG. Neonatology: pathophysiology & management of the newborn. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 1235-47.

8   Chiesa C, Pellegrini G, Panero A, et al. C-reactive protein, interleukin–6, and Procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. *Clin Chem* 2003; 49: 60-8.

9   Ng PC, Lam HS. Diagnostic markers for neonatal sepsis. *Curr Opin Pediatr* 2006; 18: 125-31.

10   Yoon BH, Romero R, Shim JY, et al. C-reactive protein in umbilical cord blood: a simple and widely available clinical method to assess the risk of amniotic fluid infection and funisitis. *J Matern Fetal Neonatal Med* 2003; 14: 85-90.

11   Arnon S, Litmanovitz I, Regev R, et al. The prognostic virtue of inflammatory markers during late onset sepsis in preterm infants. *J Perinat Med* 2004; 32: 176-80.

12   Chiesa C, Signore F, Assumma M, et al. Serial measurement of C–reactive protein and interleukin–6 in the immediate postnatal period: reference intervals and analysis of maternal and perinatal confounders. *Clin Chem* 2001; 47: 1016-22.

13   Dollner H, Vatten L, Austgulen 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: 1251-7.

14   Mathai E, Christopher U, Mathai M, et al. Is C-reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection? *Indian Pediatr* 2004; 41: 895-900.

15   Rite Gracia S, Grasa Ullrich JM, Ruiz de la Cuesta Martín C, et al. Interleukin–6 and tumor necrosis factor–alpha as markers of vertically transmitted neonatal bacterial infection. *An Pediatr (Barc)* 2003; 59: 246-51.

16   Philip AG, Mills PC. Use of C–reactive protein in minimizing antibiotic exposure. experience with infants initially admitted to a well-baby nursery. *Pediatrics* 2000; 106: E4.

17   Içağasioğlu D, Caksen H, Sütçu I, Cevit O. Serum C–reactive protein and interleukin–6 levels in neonatal sepsis. *Acta Medica (Hradec Kralove)* 2002; 45: 111-3.

18   Puopolo KM. Bacterial and fungal infections. In: Cloherty JP, Eichenwald, Stark AR. Manual of neonatal care. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 287-97.

19   Krueger M, Nauck MS, Sang S, et al. Cord blood levels of interleukin–6 and interleukin–8 for the immediate diagnosis of early onset infection in premature infants. *Biol Neonate* 2001; 80: 118-23.

20   Schultz C, Temming P, Bucsky P, et al. Immature anti–inflammatory response in neonates. *Clin Exp Immunol* 2004; 135: 130-6.

21   Volante E, Moretti S, Pisani F, Bevilacqua G. Early diagnosis of bacterial infection in the neonate. *J Matern Fetal Neonatal Med* 2004; 16: 13-6.

22   Laborada G, Rego M, Jain A, et al. Diagnostic Value of cytokines and C-reactive protein in the first 24 hours of neonatal sepsis. *Am J Perinatol* 2003; 20: 491-501.

23   Døllner H, Vatten L, Linnebo I, et al. Inflammatory mediators in umbilical plasma from neonates who develop early onset sepsis. *Biol Neonate* 2001; 80: 41-7.

24   Dollner H, Arntzen KJ, Haereid PE, et al. Interlekin–6 Concentrations in neonates evaluated for sepsis. *J Pediatr* 1998; 132: 295-9.

25   Resch B, Gusenleitner W, Müller WD. Procalcitonin and interleukin–6 in the diagnosis of early onset sepsis of the neonate. *Acta Paediatr* 2003; 92: 243-5.