An Optimal Cut-off Point of Serum C-Reactive Protein in Prediction of Neonatal Sepsis

Suppawat Boonkasidecha MD*,
Jantana Panburana MD*, Somboon Chansakulporn MD*,
Banchaun Benjasuwantep MD*, Kittipong Kongsomboon MD**

** Department of Pediatrics, Faculty of Medicine, Srinakarinwirot University, Nakhon Nayok, Thailand*
*** Department of Preventive and Social Medicine, Faculty of Medicine, Srinakarinwirot University, Nakhon Nayok, Thailand*

Objective: *To determine an optimal cut-off point of serum C-reactive protein (CRP) levels for prediction of neonatal sepsis.*
Material and Method: *A prospective cohort study of neonates aged from birth to 30 days old presenting with signs and symptoms of neonatal sepsis in neonatal intensive care unit (NICU) from January 2010 through December 2011 was performed. Neonates were assigned to either sepsis or normal group depending on blood culture status. Serial CRP (12-24 hours apart) and complete blood count were then analyzed using independent t-test, Wilcoxon rank-sum test and Receiver operating characteristic (ROC) curves.*
Results: *Of 53 neonates recruited into the present study, 26 (49%) were assigned to sepsis group and the remaining 27 (51%) were assigned to normal group. Baseline characteristics for the two groups were similar except for the higher amount of male participants in sepsis group (p-value 0.006). Most patients in sepsis group (7/26) demonstrated coagulase-negative staphylococci (CoNS) sepsis. The values of 1st CRP and 2nd CRP were significantly higher in sepsis group compared to normal group (p-value < 0.001 and 0.003). From ROC curves, at the cut-off points of 1st CRP $\geq$ 1.90 mg/L and 2nd CRP $\geq$ 1.25 mg/L, the sensitivity were as high as 92.6% and 96.3%, respectively, and the specificity were both at 100%.*
Conclusion: *Serial CRP is safe as diagnostic tool to consider antimicrobial treatment in neonatal sepsis with sensitivity of 92.6% and 96.3% for the first CRP cut-off point $\geq$ 1.90 mg/L and the second CRP $\geq$ 1.25 mg/L with 100% positive predictive value. Moreover, these safety profiles might help in reducing overuse of antibiotics with negative predictive value 96.3%.*

Keywords: *Neonatal sepsis, Neonatal infection, C-reactive protein (CRP)*

J Med Assoc Thai 2013; 96 (Suppl. 1): S65-S70
Full text. e-Journal: *http://jmat.mat.or.th*

Neonatal sepsis remains a major life-threatening condition despite advanced medical development. The incidence of early onset neonatal sepsis in USA is about 1.5% of live births in 2002[1,2]. The definite diagnosis obtained by blood culture is delayed, posing difficulty in guiding antimicrobial treatment and duration which might lead to overuse of antibiotics. Fanaroff et al reported that signs and symptoms associated with neonatal sepsis were non-specific and had low positive predictive values only 13-20%[3]. Serial C-reactive protein (CRP) is utilized in aiding diagnosis of neonatal sepsis with high sensitivity and specificity. Predictive values of CRP for neonatal sepsis were reported approximately 75-85% for sensitivity and specificity at the cut-off point 5-10 mg/L in many studies[4-10]. The objectives of the present study were to determine the optimal cut-off point of CRP and assess the predictive values of the new optimal CRP level from the present study as the diagnostic test in neonatal sepsis.

Correspondence to:
Boonkasidecha S, Neonatal Division, Department of Pediatrics, Faculty of Medicine, Srinakarinwirot University, Ongkharak, Nakhon Nayok 20120, Thailand.
Phone: 037-395-085
E-mail: suppawat_yoo@hotmail.com

Material and Method

A prospective cohort study of neonates aged from birth to 30 days old presenting with signs and symptoms of neonatal sepsis in NICU and nursery ward of Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center, Department of Pediatrics, Srinakharinwirot University. Neonates were assigned to either sepsis group or normal group depending on blood culture status. Haematogical parameters from complete blood count and serial CRP were then analyzed using independent t-test Wilcoxon rank-sum test and Receiver operating characteristic (ROC) curves. The study protocol was approved by the institutional review board and informed consent was obtained from each

infant's parents.

Population included all newborn infants, who presented with signs and symptoms of neonatal sepsis, e.g. thermoregulation instability, lethargy, apnea, respiratory distress, abdominal distension, increasing oxygen requirement or respiratory support, metabolic derangement. Infants who had some conditions such as postoperative PDA ligation, intracranial hemorrhage, post resuscitation from severe asphyxia and antibiotics given before sepsis work-up were excluded. Infants who met the criteria will have the completed sepsis work-up done including complete blood count (CBC), blood culture, urinalysis, urine culture and cerebrospinal fluid analysis. Radiographic studies for localized infections were performed as clinically indicated. One and a half mL of blood was required for a serum CRP measurement which was performed by using a commercial kit CRP (Latex) US, Roche Diagnostics Corporation, Indianapolis, IN, USA). CRP level was obtained at time of initial sepsis work-up and again at 12-24 hours later. Final diagnosis based on blood culture results.

Results

During the present study period, 72 neonates who were admitted with suspicious signs for sepsis. However, 19 infants were excluded due to severe asphyxia (7 cases), intracranial hemorrhage (6 cases), postoperative PDA ligation (3 cases), antibiotics given before completed sepsis work up (2 cases) and parents refusal (1 case) respectively. Of 53 neonates recruited into the study, 26 (49%) were assigned to sepsis group and the remaining 27 (51%) were assigned to normal group. Baseline characteristics for the two groups were similar except for the higher amount of male participants in sepsis group (p-value 0.006). Most patients in sepsis group (7/26) demonstrated coagulase-negative staphylococci (CoNS) sepsis the other organisms were shown in Table 2. The values of 1st CRP and 2nd CRP were significantly higher in sepsis group compared to normal group (p-value < 0.001 and 0.003, respectively).

Patients in sepsis group illustrated significantly lower absolute neutrophil counts and lower platelet counts < 150,000/mm^3 along with higher I:T

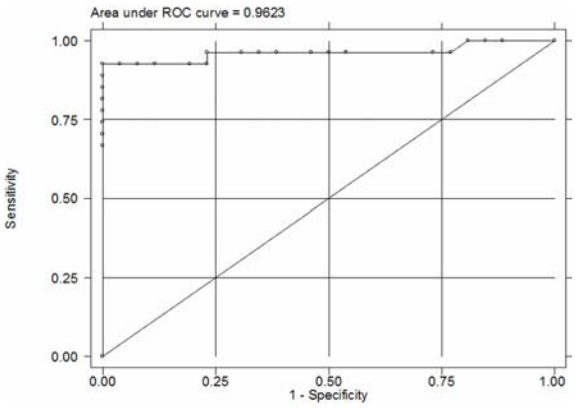

Fig. 1 Demonstration cut-off point and area under ROC curve of 1st CRP ≥ 1.90 mg/L

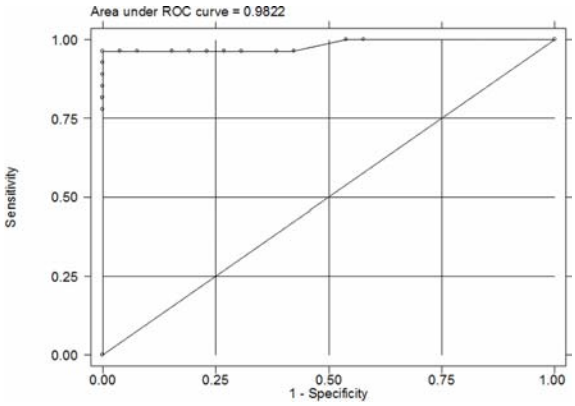

Fig. 2 Demonstration cut-off point and area under ROC curve of 2nd CRP ≥ 1.25 mg/L

Table 1. Characteristics of 53 neonates divided to normal and sepsis group

Parameters	Normal Group (Mean ± SD)	Sepsis Group (Mean ± SD)	p-value
Number	27	26	-
Sex: male (%)	13 (48.1)	23 (85.1)	0.006*
Gestation age(weeks)	34 ± 3.8	34 ± 3.4	0.97**
Birth weight (grams)	2,200.6 ± 1,043.1	2,077.3 ± 859.7	0.64**
Age of onset (days)	10.5 ± 8.1	9.15 ± 8.2	0.67**
Caesarean section (%)	12 (52.2)	11 (47.8)	0.69*
Rupture of membrane (hours)	8.6 ± 2.1	12.5 ± 8.1	0.22*

* Chi-square test, ** Independent t-test

ratio > 0.16, I:M ratio > 0.25 and band form finding. From ROC curves, at the cut-off points of 1st CRP ≥ 1.90 mg/L and 2nd CRP ≥ 1.25 mg/L, the sensitivity were as high as 92.6% and 96.3%, respectively, and the specificity were both at 100%. It clearly showed that serial CRP at 12 to 24 hours after the onset of infection had very high negative predictive values of 92.9% and 96.3% and positive predictive value of 100%.

Discussion

Neonatal septicemia is one of the major causes of death in neonatal period. To establish the diagnosis is difficult due to non-specific symptoms and signs[1-3]. Initiate treatment with empirical antibiotics as soon as suspected infection is not uncommon condition. The diagnosis obtained by blood culture is delayed which might lead to overuse of antibiotics[4-11]. This clinical practice could result in an increase incidence of antibacterial resistant organisms and a high medical cost. Thus, a sensitive diagnostic test which differentiates infected from non-infected neonates would be very useful. In the present study, the authors found the new cut-off point of CRP that lower level than discovered in the past. The results showed the 1st CRP ≥ 1.90 mg/L and 2nd CRP ≥ 1.25 mg/L had very high sensitivity, specificity and positive predictive value. The advantages of CRP over the parameters from CBC are that it does not depend on gestational age, post-natal age, hemoglobin level, or red blood cell mass and because it does not cross the placenta, therefore increased CRP level indicates the response of the newborns by themselves to infection or inflammation[5,7-9].

On the other hand, false positive CRP had been reported in several studies. The increased CRP in non infective condition can occurred in the infants who already had tissue injury e.g. postoperative PDA

Table 2. Causative organisms in the sepsis group

Organisms	Number
Coagulase-negative staphylococci	7
Klebsiella pneumoniae	4
Enterobacter cloacae	2
Acinetobacter baumanii	2
Group B streptococcii	2
Escherichia coli	2
Pseudomonas aeruginosa	2
Candida albicans	2
Enterobacter faecii	1
Proteus mirabilis	1
Herpes simplex	1

Table 3. The first and second CRP results in the normal and sepsis group

Parameters	Normal group median (inter-quartile range)	Sepsis group median (inter-quartile range)	p-value*
1st CRP (mg/L)	0.27 (0.15-0.80)	30.40 (10.23-79.64)	< 0.001
2nd CRP (mg/L)	0.10 (0-0.30)	21.40 (8.70-47.75)	< 0.001

* Wilcoxon rank-sum test

Table 4. The hematologic parameters of CBC in the normal and sepsis group

Paraneters	Normal group	Sepsis group	p-value
Total WBC, mean ± SD	15,151.81 ± 6,421.39	11,647.70 ± 7,117.52	0.07*
Absolute neutrophil counts, mean ± SD	8,156.77 ± 4,678.55	4,991.59 ± 4,156.54	0.01*
I:T ratio, median (inter-quartile range)	0.05 (0-0.13)	0.23 (0.10-0.33)	< 0.001*
I:M ratio, median (inter-quartile range)	0.11 (0-0.22)	0.25 (0.11-0.43)	0.01**
Band form (%), median (inter-quartile range)	0 (0-1)	1 (0-2)	0.006**
Platelet counts, mean ± SD	204,132.70 ± 79,694.54	142,231.50 ± 117,327.10	0.03*

* Independent t-test, ** Wilcoxon rank-sum test
I:T ratio: immature/total neutrophil
I:M ratio: immature/mature neutrophil

Table 5. The predictive values of the first and second CRP results in sepsis screening

Parameters	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
1st CRP*	92.6	100	92.9	100
2nd CRP **	96.3	100	96.3	100

* Cut-off point for 1st CRP ≥ 1.90 mg/L, ** Cut-off point for 2nd CRP ≥ 1.25 mg/L
NPV = negative predictive value, PPV = positive predictive value

ligation, intracranial hemorrhage and post resuscitation from severe asphyxia[7,11]. Physicians can predict the events associated with elevated CRP and can be clinically aware of the possibility of false positive CRP. Even so, we did not find false positive CRP in the present study. Because neonatal sepsis remains a major life-threatening condition and the yield of positive bacterial culture is low and delayed. Most of the suspected sepsis infants have mild symptoms and should be treated base on maternal risk factors and clinical manifestations[10,11].

Our finding supports to use serial CRP instead of a single measurement as in the past studies[12-16]. In addition, due to the higher negative predictive values and sensitivity of the second CRP than the first CRP, physicians might obtain only one CRP measurement at 24 to 48 hours after the onset of suspected sepsis as a diagnostic test if there is the limited budget situation or lack of blood culture facility.

From the present study, due to very high NPV of CRP, practitioners can consider using a negative CRP which level < CRP 1.90 mg/L at the first time of initial sepsis work-up and level < CRP 1.25 mg/L at 12-24 hours later together with clinical evaluation to cease the unnecessary antimicrobials that would help to reduce cost, hospital stay and decrease the risk of developing resistant organisms in NICU[17,18].

In conclusion, the cut-off points of CRP levels, obtained from the present study, are lower than the results from previous studies. Nevertheless, there was asymmetrical distribution of the causative organisms of neonatal sepsis, which CoNS has been found more than other organisms[18,19] and this might bring about the lower cut-off point levels compared with previous studies. To provide the exact cut-off point of CRP level, subanalysis of the CoNS group, separated from the others, may get more appropriate cut-off point of CRP level in future studies.

Potential conflicts of interest

None.

References

1. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N Engl J Med 2002; 347: 240-7.
2. Klinger G, Levy I, Sirota L, Boyko V, Reichman B, Lerner-Geva L. Epidemiology and risk factors for early onset sepsis among very-low-birthweight infants. Am J Obstet Gynecol 2009; 201: 38.e1-6.
3. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics 2002; 110: 285-91.
4. Fanaroff AA, Korones SB, Wright LL, Verter J, Poland RL, Bauer CR, et al. Incidence, presenting features, risk factors and significance of late onset septicemia in very low birth weight infants. The National Institute of Child Health and Human Development Neonatal Research Network. Pediatr Infect Dis J 1998; 17: 593-8.
5. Philip AG, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980; 65: 1036-41.
6. Dyck RF, Bingham W, Tan L, Rogers SL. Serum levels of C-reactive protein in neonatal respiratory distress syndrome. Clin Pediatr (Phila) 1984; 23: 381-3.
7. Nakamura H, Uetani Y, Nagata T, Yamasaki T. Serum C-reactive protein in the early diagnosis of neonatal septicemia and bacterial meningitis. Acta Paediatr Jpn 1989; 31: 567-71.
8. Schouten-Van Meeteren NY, Rietveld A, Moolenaar AJ, Van Bel F. Influence of perinatal conditions on C-reactive protein production. J Pediatr 1992; 120: 621-4.
9. Pourcyrous M, Bada HS, Korones SB, Baselski V, Wong SP. Significance of serial C-reactive protein responses in neonatal infection and other disorders. Pediatrics 1993; 92: 431-5.
10. Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the

diagnosis of neonatal infection. Pediatrics 1998; 102: E41.
11. Jaye DL, Waites KB. Clinical applications of C-reactive protein in pediatrics. Pediatr Infect Dis J 1997; 16: 735-46.
12. 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.
13. Hengst JM. The role of C-reactive protein in the evaluation and management of infants with suspected sepsis. Adv Neonatal Care 2003; 3: 3-13.
14. Malik A, Hui CP, Pennie RA, Kirpalani H. Beyond the complete blood cell count and C-reactive protein: a systematic review of modern diagnostic tests for neonatal sepsis. Arch Pediatr Adolesc Med 2003; 157: 511-6.
15. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003; 111: 1805-12.
16. Philip AG, Baker CJ. Cerebrospinal fluid C-reactive protein in neonatal meningitis. J Pediatr 1983; 102: 715-7.
17. Turner MA, Power S, Emmerson AJ. Gestational age and the C reactive protein response. Arch Dis Child Fetal Neonatal Ed 2004; 89: F272-F273.
18. van den Hoogen A, Gerards LJ, Verboon-Maciolek MA, Fleer A, Krediet TG. Long-term trends in the epidemiology of neonatal sepsis and antibiotic susceptibility of causative agents. Neonatology 2010; 97: 22-8.
19. Lin FY, Weisman LE, Azimi P, Young AE, Chang K, Cielo M, et al. Assessment of intrapartum antibiotic prophylaxis for the prevention of early-onset group B Streptococcal disease. Pediatr Infect Dis J 2011; 30: 759-63.

การใช้ค่า C-reactive protein ที่เหมาะสมในการทำนายการติดเชื้อในกระแสเลือดของทารกแรกเกิด

ศุภวัชร บุญกษิดิ์เดช, จันทนา พันธ์บูรณะ, สมบูรณ์ จันทร์สกุลพร, บานชื่น เบญจสุวรรณเทพ, กิตติพงษ์ คงสมบูรณ์

วัตถุประสงค์: *เพื่อกำหนดและประเมินค่า C-reactive protein (CRP) ที่เหมาะสมในการคัดกรองและทำนายการติดเชื้อในกระแสเลือด โดยเปรียบเทียบกับการเพาะเชื้อในกระแสเลือดเพื่อยืนยันภาวะการติดเชื้อในกระแสเลือดของทารกแรกเกิด*

วัสดุและวิธีการ: *เป็นการศึกษาแบบไปข้างหน้าโดยติดตามทารกตั้งแต่แรกเกิดจนถึงอายุ 30 วัน ที่สงสัยว่ามีภาวะติดเชื้อในหน่วยทารกแรกเกิด โรงพยาบาลศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี เริ่มจากเดือนมกราคม พ.ศ. 2553 ถึงเดือนธันวาคม พ.ศ. 2554 โดยมีการเก็บตัวอย่างเลือด จากทารกที่สงสัยภาวะเชื้อในกระแสเลือดภายใน 12-24 ชั่วโมงแรก ก่อนให้ยาปฏิชีวนะ โดยเลือดที่ได้จะทำการส่งเพาะเชื้อในกระแสเลือด ตรวจนับเม็ดเลือดและระดับ CRP 2 ครั้งห่างกัน 12-24 ชั่วโมง แบ่งทารกเป็น 2 กลุ่ม จากผลการเพาะเชื้อในกระแสเลือดที่ได้และทำการวิเคราะห์ทางสถิติโดยใช้ independent t-test Wilcoxon rank-sum test และใช้กราฟ ROC*

ผลการศึกษา: *ทารกเข้าสู่การศึกษาทั้งหมด 53 ราย ในจำนวนนี้มีผลบวกต่อการเพาะเชื้อในกระแสเลือดทั้งหมด 26 ราย คิดเป็นร้อยละ 49 (กลุ่ม sepsis) โดยพบปัจจัยเสี่ยงที่มีนัยสำคัญคือ เพศชาย และชนิดของเชื้อที่พบมากที่สุดคือ coagulase-negative Staphylococci 7 ราย คิดเป็นร้อยละ 27 ค่า cut-off point ที่เหมาะสมของ CRP ครั้งที่ 1 และครั้งที่ 2 คือ ≥ 1.90 และ ≥ 1.25 มก./ล. ตามลำดับ ซึ่งมีความไวของการทดสอบต่อภาวะการติดเชื้อในกระแสโลหิตสูงถึงร้อยละ 92.6 และ 96.3 ตามลำดับ ขณะที่ค่า negative predictive value (NPV) ของ CRP1 และ CRP2 มีค่าสูงถึงร้อยละ 92.9 และ 96.3 ตามลำดับ โดยมีความจำเพาะของการทดสอบร้อยละ 100 ทั้งสองค่า*

สรุป: *การใช้ค่า C-reactive protein (CRP) 2 ครั้งห่างกัน 12-24 ชั่วโมงมีความไวและความจำเพาะของการทดสอบสูงในการพิจารณาตัดสินใจให้การรักษาทารกที่สงสัยการติดเชื้อในกระแสโลหิต โดยจากการศึกษานี้ พบค่า cut-off point ที่เหมาะสมของ CRP ครั้งที่ 1 และครั้งที่ 2 คือ ≥ 1.90 และ ≥ 1.25 มก./ล. ซึ่งมีความไวในการทำนายภาวะการติดเชื้อในกระแสเลือดสูงถึงร้อยละ 92.6 และ 96.3 ตามลำดับ ขณะที่มีค่า negative predictive value สูงถึงร้อยละ 96.3*