

Strong Positive Correlation between Neutrophil-to-lymphocyte Ratio and C-reactive Protein in Early Onset Sepsis

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Abstract

Sepsis is a life-threatening disease with a high number of mortality in premature infants. Premature infants have immature immune systems, with less pool neutrophils and imperfect ability to destroy pathogen. Neutrophil function is supported by lymphocyte's ability to form antibody or specific cell-surface receptors for particular antigens. This underlies the use of neutrophil-to-lymphocyte ratio (NLR) as an inflammation marker to detect and assess the severity of sepsis. C-reactive protein (CRP) is known as an acute phase reactant. Neutrophil-to-lymphocyte ratio is an easier, fast, and inexpensive method when compared to CRP. The aim of this study was to evaluate the correlation between NLR and CRP in detecting early-onset sepsis (EOS) in premature infants. A cross-sectional study was conducted on 53 premature infants born and hospitalized in a hospital in Indonesia who were recruited during the period of April to October 2018. Blood was sampled from the umbilical cord at birth for laboratory examination. The NLR was determined as the ratio of neutrophil to lymphocyte count. The Tollner scoring system was used to identify sepsis. Mann Whitney-U test and Spearman Correlation test were computed for the statistical analysis. neutrophil-to-lymphocyte ratio which results showed a strong positive correlation with CRP ($r=0.702$, $0=0.001$) in premature infants with EOS. Leukocyte count was lower in infants with EOS than those without EOS group (median; IQR, $\times 10^3$: 8.9 (6.3–13.8) vs 12.5 (10.1–16.1); $p=0.016$). Neutrophil-to-lymphocyte ratio and CRP tended to be lower in EOS group when compared to that of the non-EOS group. In conclusion, NLR has a strong positive correlation with CRP in premature infants with EOS.

Key words: C-reactive protein, early-onset sepsis, neutrophil-to-lymphocyte ratio, premature infant

Korelasi Positif Kuat antara *Neutrophil-to-lymphocyte* dan *C-reactive Protein* pada Sepsis Awitan Dini

Abstrak

Sepsis pada bayi prematur adalah keadaan mengancam jiwa dengan mortalitas tinggi. Bayi prematur memiliki sistem imun imatur, sedikit persediaan neutrofil serta fungsi belum sempurna untuk melawan patogen. Fungsi neutrofil didukung limfosit membuat antibodi serta reseptor permukaan spesifik untuk antigen tertentu. *Neutrophil-to-lymphocyte ratio* (NLR) diajukan untuk petanda inflamasi, mendeteksi dan menentukan derajat sepsis, sedangkan *C-reactive protein* (CRP) telah diketahui sebagai protein fase akut. *Neutrophil-to-lymphocyte ratio* adalah pemeriksaan yang lebih mudah, cepat, murah dibanding dengan CRP. Penelitian potong lintang ini bertujuan mengevaluasi korelasi NLR dengan CRP untuk mendeteksi sepsis awitan dini pada 53 bayi prematur, yang lahir dan dirawat di rumah sakit kami, selama April–Oktober 2018. Sampel darah dari tali pusat pada saat lahir. *Neutrophil-to-lymphocyte ratio* adalah rasio jumlah neutrofil terhadap limfosit. Skoring Tollner digunakan untuk mengidentifikasi sepsis. Analisis statistik menggunakan Uji Mann-Whitney dan korelasi Spearman. Korelasi positif antara NLR dengan CRP di antara bayi prematur dengan sepsis awitan dini adalah $r=0,702$, $p=0,001$. Bayi dengan sepsis awitan dini memiliki jumlah leukosit lebih rendah dibanding bayi non-sepsis (median; IQR, $\times 10^3$: 8,9 (6,3–13,8) vs 12,5 (10,1–16,1); $p=0,016$). *Neutrophil-to-lymphocyte ratio* dan CRP lebih rendah pada kelompok sepsis dibanding non-sepsis. Simpulan, NLR memiliki korelasi positif kuat dengan CRP pada bayi prematur dengan sepsis awitan dini.

Kata kunci: Bayi prematur, *C-reactive protein*, *neutrophil-to-lymphocyte ratio*, sepsis awitan dini

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Introduction

Sepsis is the most frequent cause of neonatal death in developing countries. Sepsis in premature infants manifests in nonspecific signs and symptom that determination of sepsis is still a challenge in low resource settings.^{1, 2} Premature infants are born with an immature immune system and have an innate immune system with low response to infection due to the lack of soluble peptide and cellular responses.¹ The lower number of monocytes and neutrophils in premature infants also leads to relative neutropenia and monocytopenia, as well as fewer cytokines.³

Neutrophils are the first immune cells that respond to bacterial, viral, and fungal infections. Neutrophil count will increase and these cells will move to the infected region and perform their functions such as phagocytosis, formation of oxygen radicals, and intracellular killing of pathogens. Neutrophils also discharge cytokine and activate T-cell.⁴ Along with the phenomenon, a rise in anti-inflammatory cytokines will lead to immunosuppression, and induce apoptosis of a certain number of lymphocytes. Thus, lymphopenia state is an indicator of immunosuppression that may lead to sepsis.⁵

A low absolute numbers of circulating lymphocytes is seen in premature infants, which will lead to clonal expansion with most are naïve T cells.⁶ This condition is a result of incompetent dendritic cell (DC) function during antigen uptake and presentation and inefficient MHC II expression of the antigen-presenting cells.⁷

C-reactive protein (CRP) is classified as a major acute-phase protein that plays a central role in innate and adaptive immunity. The level of CRP increases in response to inflammation which becomes the reason for the use of CRP as one of the frequently used markers to define neonatal sepsis.⁸ Since CRP increases in several clinical conditions, other laboratory tests are needed to determine diagnosis. The most frequently used laboratory tests to support diagnosis of neonatal sepsis are white blood cell count, immature-to-total neutrophil ratio, and absolute neutrophil count.

Neutrophil-to-Lymphocyte Ratio (NLR) has been proposed as a simple biomarker of systemic inflammation in neonates; however, data on the use of NLR to determine neonatal sepsis are still scarce.⁹ The objective of this study was to investigate the correlation between NLR and CRP in suspected early-onset sepsis (EOS) cases.

Methods

This was a cross-sectional study conducted at the Neonatology Division, Department of Child Health Faculty of Medicine Universitas Padjadjaran Dr. Hasan Sadikin General Hospital Bandung, Indonesia, from April to October 2018. The inclusion criteria were neonates with 28 to 34-week gestational age who had data on CBC examination at birth. A total of 80 infants (28–34 weeks of gestational age) were included in the study through consecutive sampling. Of this number, only 53 infants had data on CBC examination at birth and only 45 infants had been examined for CRP.

Infants with a major congenital anomaly, twins, and who were born from infected or diabetic mother were excluded from the study. Verbal consent from either parents or caregiver was obtained before enrolment in the study.

Subjects then underwent thorough history taking and comprehensive clinical examination to determine the gestational age using new Ballard score as well as to identify signs of respiratory distress, mode of delivery, and birth weight. Early-onset sepsis was identified using the Tollner scoring.^{9,10}

Cord blood was collected from each eligible participant. A total of 3 mL cord blood were collected using the local standard procedure and the blood was directly processed in the laboratory to examine the CBC and CRP. Complete blood count was performed using automated flow cytometry hematology analyzer (Sysmex XN series, Sysmex Corp., Japan). The ratios of circulating neutrophil and lymphocyte, which was marked by NLR, were calculated using the absolute count of each parameter from complete blood count. The serum CRP level was measured using the particle-enhanced turbidimetric immunoassay (PEITA) method (Siemens Dimensions Series).

Collected data were computed using Statistical Package for Social Science (SPSS version 23 for Windows; SPSS Inc, Chicago). The primary analysis of this study aimed to assess the differences in clinical manifestations and laboratory parameters between the two observation groups. This analysis was performed using Mann-Whitney U test. The second analysis was operated to analyze the relationship between NLR and CRP level, stratified by EOS and non-EOS group. This analysis was approached by a statistical calculation from Spearman correlation. A significant result was defined as

p-value of less than 0.05 (<0.05). Analyzed data were presented as a distribution frequency table and graphs.

Töllner’s sepsis score (TSS) was computed for each subject (Tollner, 1982). The TSS consists of clinical parameters (skin colour, body temperature, muscle tone, breath rate, abdominal distension, defected microcirculation, and risk factors) and laboratory parameters (leukocyte and thrombocyte counts, immature/total neutrophil ratio, CRP). A score was assigned for each parameter (0, 1, 2, or 3) based on the condition found according to its severity. For example, 0 for normal muscle tonus, 1 for hypotonia, 2 for flask tonus, 0 for normal leukocyte count, 1 for leukocytosis, and 3 for leukopenia. Based on the result of the TSS, subjects who had a score of >10 points were identified as experiencing neonatal sepsis.⁹

All procedures were conducted following the policies of the Faculty of Medicine Universitas Padjadjaran and Dr. Hasan Sadikin General Hospital Bandung, West Java, Indonesia. This study was approved by the Health Research Ethics Committee of Faculty of Medicine Universitas Padjadjaran Bandung with the issuance of the ethical clearance number 303/UN6.KEP/EC/2018. Consent was obtained for all participants.

Results

The 53 premature infants included in the study were classified into 2 groups: EOS (22 infants) and non-EOS (31 infants). The demographic and clinical characteristics of each group and their exposure to therapy are presented in Table 1. Birth weight and gestational age of EOS group was significantly lower than those of non-EOS group. The two groups did not differ significantly with regards to gender distribution and delivery method. There were no babies born with APGAR less than 3 at five minutes after delivery in both of group. Infants with antenatal corticosteroid showed a risk for EOS 3.5 times higher when compared to the other group.

The comparison of hematologic parameters at birth between EOS and non-EOS infants is displayed in Table 2. At birth, there was no difference in hemoglobin levels between the EOS and non-EOS groups. The total leukocyte count of EOS group was significantly lower than that of non-EOS group. The same applies to white blood cell line (neutrophil, eosinophil, monocyte, lymphocyte and basophil counts) which tended to be lower in the EOS group than the non-EOS group, albeit insignificantly. The NLR and CRP showed the same pattern as the hematology parameters, which tended to be lower in EOS

Table 1 Comparison of Baseline Parameters of Premature Infants With and Without Early Onset Sepsis

Variable	Total Population n=53	EOS n=22	Non-EOS n=31	p*
Birth Weight (grams mean±SD)		1502.73±294.93	1757.42±296.94	0.003
Gestational age (weeks±SD)		31.5±2.18	32.7±1.7	0.038
Gender				
Male	31(58.5%)	13(41.9%)	18(58.1%)	0.940
Female	22(41.5%)	9(40.9%)	13(59.1%)	
Antenatal corticosteroid				0.041
Yes	16(30.2%)	10(62.5%)	6(37.5%)	OR=3.5; 95%CI (1.0-11.8)
No	37(69.8%)	12(32.4%)	25(67.6%)	
Delivery method				
CS	34(64.2%)	12(35.3%)	22(64.7%)	0.219
Vaginal delivery	19(35.8%)	10(52.6%)	9(47.4%)	

*Chi-Square test; CS: caesarean section, EOS: early-onset neonatal sepsis

Table 2 Comparison of Blood Parameters of Premature Infants With and Without Early Onset Sepsis

Variable (Median, IQR)	EOS	NON-EOS	P ^a
Hemoglobin (g/dL)	17.1 (15.1-20.0)	16.7 (13.6-19.4)	0.588
Leukocyte (10 ³ /μL)	8.9 (6.3-13.8)	12.5 (10.1- 16.1)	0.016
Basophil (10 ³ /μL)	0.03 (0.01-0.05)	0.03 (0.02-0.07)	0.259
Eosinophil (10 ³ /μL)	0.14 (0.08-0.31)	0.16 (0.05-0.26)	0.704
Neutrophil (10 ³ /μL)	4.59 (2.34-8.69)	7.36 (6.33-9.33)	0.035
Lymphocyte (10 ³ /μL)	2.96 (2.17-3.38)	3.17 (2.36-4.11)	0.274
Monocyte (10 ³ /μL)	1.08 (0.61-2.00)	1.37 (1.15-1.97)	0.111
NLR	1.57 (0.68-3.30)	2.26 (1.27-3.93)	0.126
CRP (mg/dL)	0.11 (0.06-0.27)	0.15 (0.09-0.22)	0.269

^aMan Whitney U-test; EOS: Early Onset Sepsis; NLR: neutrophil-to-lymphocyte ratio; CRP: C-Reactive Protein

than in non-EOS groups.

The performance of NLR and CRP levels in predicting sepsis in premature infants was evaluated using the ROC curves (Figure 2). The NLR and CRP levels presented poor performance in predicting EOS (NLR: AUC =0.365, p=0.131;

CRP levels: AUC =0.354, p=0.103). The NLR had similar performance with the CRP level (Figure 1).

A strong positive correlation between NLR and CRP at birth was identified in premature infants with EOS with $r=0.702, p=0.001$.

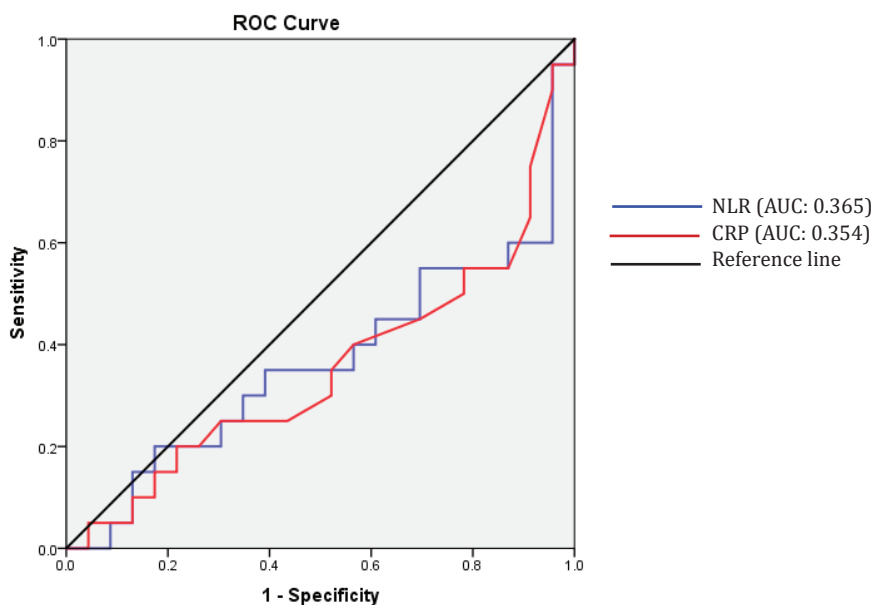


Figure 1 Receiver Operating Characteristic Curves of NLR and CRP in Predicting EOS in Premature Infants

Areas under the receiver operating characteristic (ROC) curves: NLR (blue line): 0.365 (95% confidence interval (CI): 0.192 to 0.538, p=0.131); the CRP level (red line): 0.354 (95% CI: 0.184 to 0.524, p=0.103)

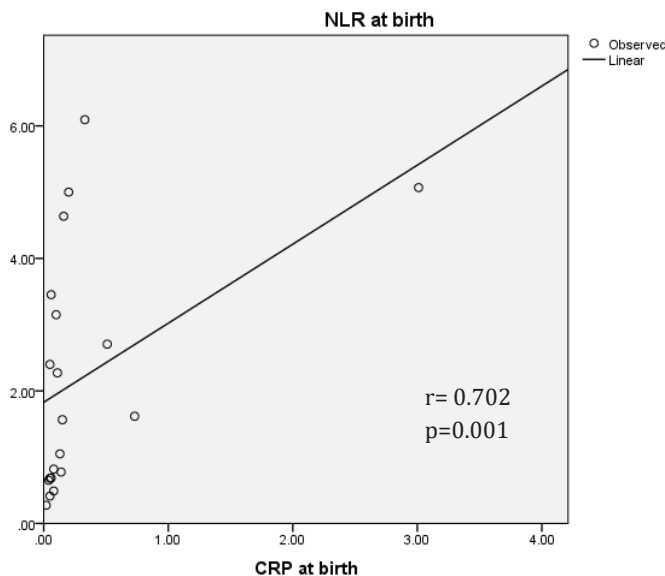


Figure 2 Correlation between Neutrophil-to-Lymphocyte Ratio and C-reactive Protein at Birth among Premature Onfants with EOS

Discussion

Premature infants from EOS group showed lower birth weight and gestational age when compared to infants in the non-EOS group (Table 1). This is in agreement with the physiology of neonates that infants with lower gestational age are more susceptible to sepsis.¹ On the other hand, birth weight increased with gestational age in premature infants.¹² Table 1 demonstrates that premature infants with antenatal corticosteroid had a 3.5 times higher risk to EOS when compared to the group without antenatal steroid. Antenatal corticosteroid treatment is commonly given to premature infants to reduce the risk of neonatal respiratory disease. This treatment triggers an immunosuppressive condition and has been correlated with minimum lymphocyte proliferation and cytokine release as well as increased risk of infection.¹ In addition, the total leukocyte count and white blood cell line of the EOS group was found to be lower when compared to the non-EOS group. Physiologically, these facts correlate with gestational age, leading to a high rate of infection during neonatal period.⁴

With these conditions, lower NLR value was observed in the EOS group when compared to the non-EOS group. The CRP level showed the same pattern as NLR, and demonstrated a strong positive correlation with NLR in premature

infants with EOS. Neutrophil-to-Lymphocyte Ratio os proposed as a simple biomarker of systemic inflammation in neonates because this ratio can become a clue of inflammation and it can be used to predict sepsis.¹³

Neutrophils are the first immune cells that respond to bacterial, viral, and fungal infections. Lymphopenia state is an indicator of immunosuppression and may lead to sepsis.⁵ There is a low absolute numbers of circulating lymphocytes in premature infants, leading to clonal expansion with most are naïve T cells.⁶ Data on the use of NLR to identify neonatal sepsis are still scarce.⁸ Omran et al.⁸ has compared the NLR of full-term neonates with sepsis to that of healthy babies and found that NLR is higher in septic neonate. In adult studies, NLR has been used to identify and determine sepsis severity.¹⁴⁻¹⁶

The results of this study demonstrate the poor performance of NLR and CRP levels in predicting EOS (NLR: AUC =0.365 p=0.131; CRP levels: AUC =0.354, p=0.103; Figure 1). This is contrary to the results in studies by Alkan et al. and Omran et al, that performed receiver operating curve (ROC) analysis for NLR and CRP that show a good performance area under the curve (AUC) for NLR and CRP to sepsis. Alkan et al.¹³ and Omran et al.⁸ used blood culture as the gold standard of sepsis diagnosis while this study used Tollner score

to identify sepsis. The sepsis score is useful for early detection of septicemia and distinguish other differential diagnosis in neonatal period.

A strong positive correlation between NLR and CRP at birth was observed in premature infants with EOS, with $r=0.702$, $p=0.001$. NLR is known to increase in sepsis condition.¹³ A correlation was also seen between CRP increase and sepsis.¹⁷ The NLR and CRP levels are lower in premature infants with sepsis compared to those of the non-EOS group. This might be caused by the immature immune system of the premature infants. Premature infants have a low innate immune system response to infection due to defects in soluble peptide and cellular responses.¹ This weak neonatal neutrophil responses are associated with gestational age and neonatal clinical conditions with youngest and sickest neonates show the lack of neutrophil functions.⁴ On the other hand, there is a low absolute number of circulating lymphocytes in premature infants that leads to clonal expansion with most are naïve T cells.⁶

Immunosuppression of anti-inflammatories that relates to this condition is also seen while many lymphocytes undergo apoptosis. Hence, lymphopenia is a marker of immunosuppression.¹³

In conclusion, no difference is observed between NLR and CRP for EOS and non-EOS group and between the two groups. However, a strong correlation is observed between NLR and CRP in premature infants with EOS.

This study has a limitation that data on blood culture as confirmed sepsis marker are limited. Almost all blood cultures presented sterile results or normal micro floras. This is the underlying reason for the use of Tollner score to determine suspected sepsis in neonatal ward. Another limitation is that not all premature infants had their CRP checked due to the improper sample preparation that led to lysis of the sample.

With the strong positive correlation of NLR and CRP, it is suggested that NLR should be used as a marker of systemic inflammation when neonatal sepsis is suspected.

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