Neutrophil-to-Lymphocyte Ratio as an Alternative Marker of Neonatal Sepsis in Developing Countries

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ABSTRACT

Objectives: To analyze the neutrophil-to-lymphocyte ratio (NLR) as an alternative marker of neonatal sepsis.

Methods: Consecutive sampling in all inborn neonates and admitted in the Neonatal Intensive Care Unit (NICU) with clinical manifestations of neonatal sepsis included in this cross-sectional study. Neonates with congenital anomalies and the referred neonates were excluded. Complete blood count, c-reactive protein (CRP) and blood culture were the septic work up examination carried out based on local Clinical Practical Guidelines. NLR is obtained from dividing the absolute count of neutrophils from lymphocytes manually. Cut-off value of NLR is obtained using a receiver operating characteristic curve (ROC curve). Chi-Square’s Test and the Mann-Whitney U test were used for statistical analytic with significance value p<0.05

Results: The median NLR value of 104 neonates who met the inclusion and exclusion criteria is 3.63 (2.39-6.12). NLR 2.12 have the area under the curve (AUC) of 0.63 (95% CI 0.528-0.741) and 0.725 (95% CI 0.636 - 0.814) when combined with CRP 2.7 mg/dL. NLR ≥ 2.12 in clinically sepsis neonatal had almost double risk to provide positive blood culture results (RR 1.867, 95% CI 1.077-3.235; p = 0.011).
Conclusion: NLR, part of a complete blood count, can be used as an alternative marker of easy and relatively inexpensive neonatal sepsis, especially in developing countries and detection of proven neonatal sepsis to be better when combined with CRP.

Keywords: neonate, neonatal sepsis, neutrophil-to-lymphocyte ratio, NLR

Introduction

Neonatal sepsis still a major problem for neonates around the world and contributes significant morbidity and mortality neonates (term and preterm), especially in developing countries. It was a clinical syndrome that characterized by systemic infections and characterized by the isolation of pathogens in the blood (bacteremia) and occurs in infants in the first month of life. As many as 1.6 million neonates died each year due to infection and 60% of them occurred in developing countries. Neonatal sepsis is reported as 1 - 5 per 1000 live births in developed countries and a higher incidence value is reported in developing countries (10 - 50 per 1000 live births).

Routine parameters used for neonatal sepsis have varying diagnostic values such as total leukocyte count, absolute neutrophil count (ANC), immature/total neutrophil ratio (I/T ratio) and c-reactive protein (CRP). Procalcitonin, a specific marker of bacterial infection, had a high price and not always available in various health service facilities especially in rural areas. Blood culture as a gold standard markers, took a long time and often gave negative results. The neutrophil-lymphocyte ratio (NLR) ratio was an inexpensive and part of a complete blood count that does not require additional examination. Research on NLR in bacterial infections in children especially neonates was still limited. Normal NLR values in healthy neonatal or pediatric populations have only been reported once with an average NLR value of 0.52 - 0.91. Until now, no studies has been conducted in our hospital before so the purpose of this study was to analyze the NLR as an initial marker of neonatal sepsis therefore can be used in limited resource areas.

Methods

Six months (April - September 2019) observational analytic study with cross-sectional design was carried out with consecutive sampling in all inborn neonates born in Dr. Soetomo General Academic Surabaya and treated in NICU with clinically neonatal
sepsis. Complete blood count, c-reactive protein (CRP) and blood culture are carried out before giving antibiotics according to local Clinical Practice Guidelines. Suspected neonatal sepsis, as mentioned by Haque, was characterized by the presence of one or more fetal inflammatory response syndrome (FIRS) criteria in conjunction with sign and symptoms of infection. Fetal inflammatory response (FIRS) manifested by at least two of the following criteria, tachypnea (respiratory rate/ RR >60 tpm) plus either grunting/ retraction or desaturation, body temperature abnormalities (>37.9°C or <36°C), capillary refill time (CRT) >3 seconds, abnormal leukocyte count (<4000 or >34000/mm³), CRP >10mg/dL, IL-6 or IL-8 >70 pg/ml and positive 16 SrRNA genes PCR. Sign and symptoms of infection can be grouped into clinical variables (temperature instability; heart rate/ HR ≥180 or ≤100; RR >60 bpm plus grunting/ retraction or desaturation; lethargy/ altered mental status; glucose intolerance, plasma glucose >10 mmol/L; drinking intolerance), perfusion variable (CRT >3 seconds; plasma lactic acid >3 mmol/L), hemodynamic variables (blood pressure <2 SD below normal age, systolic blood pressure <50 mmHg for 1 day old and <65 mmHg for ≤1 month old); and inflammatory variables (leukocytosis >34, 000/mm³; leukopenia <4,000/mm³; immature neutrophils >10%; I/T (immature to total neutrophil) ratio >0.2; thrombocytopenia <100,000/mm³; CRP >10 mg/dL or >2 SD above normal value; procalcitonin >8.1 mg/dL or >2 SD above normal value; IL-6 or IL-8 >70 pg/ml; and positive 16 SrRNA genes PCR. Basic characteristic such as sex, gestational age, birth weight, Lubschenco score, mode of delivery, history of premature rupture of membranes, history of mother with preeclampsia/ eclampsia, history of prenatal history of steroids and neonatal sepsis onset were evaluated in this study.

Complete blood count performed by automated hematology analyzer and include white blood count (WBC) differential as evaluation of the WBC based on light scattering characteristics. The absolute neutrophil count (ANC) and absolute lymphocyte count were identified and counted in the WBC differential. The neutrophil-to-lymphocyte ratio (NLR) is obtained by dividing the ANC to the absolute lymphocytes count recorded in the medical record manually. The diagnosis of neonatal sepsis is categorized into 2 based on blood culture results, which is proven neonatal sepsis if the blood culture results are positive and suspected neonatal sepsis if negative.
Data were analyzed using SPSS and presented by median (interquartile range). Sex, gestational age, birth weight, Lubchenco score, mode of delivery, history of premature rupture of membranes, history of mothers with preeclampsia/ eclampsia, history of prenatal steroids and neonatal sepsis onset were analyzed using Chi-square’s test while total leukocyte count, ANC, absolute lymphocytes count, NLR and CRP were analyzed using Mann-Whitney U test with significance defined as p-value <0.05. The NLR cutoff value was established using a receiver operating characteristic curve (ROC curve). Ethical clearance was approved by Ethical Committee in Health Research of Dr. Soetomo General Academic Surabaya (ref. no. 1047/KEPK/III/2019).

**Results**

Number of inborn neonates admitted at NICU during the six-month study period (April - September 2019) was 492 neonates and 260 of them were suspected neonatal sepsis. Amount of 156 neonates were excluded as samples in this study because 36 neonates with congenital abnormalities and 120 neonates were referred from other hospital (outborn neonates) so that the total subjects who met the inclusion and exclusion criteria were 104 neonates (figure 1). Neonatal sepsis in this study had an incidence of 10.6% with 52 (50%) neonates categorized as proven neonatal sepsis. Gram-negative bacteria dominate by 75% as the cause of neonatal sepsis with the most bacteria is Klebsiella pneumonia ESBL (+) of 61.5%. Table 1 informs the subjects and laboratory characteristics of the subjects in this study. The median of neutrophils-to-lymphocytes ratio (NLR) (p=0.018) and CRP (p=0.001) in the proven neonatal sepsis was significantly higher than in the suspected group (figure 2).

Figure 1. Flow diagram the research design
592 neonates admitted to NICU

260 neonates with suspected neonatal sepsis

104 neonates met the inclusion and exclusion criteria

156 neonates excluded:
- 36 neonates with congenital anomaly
- 120 outborn neonates and referred to NICU

52 suspected neonatal sepsis

52 proven neonatal sepsis

260 neonates with suspected neonatal sepsis
| Table 1. Subjects Characteristic                  | Proven Neonatal Sepsis n (%) | Suspected Neonatal Sepsis n (%) | Total n (%) | p     |
|-------------------------------------------------|-------------------------------|-------------------------------|-------------|-------|
| Sex                                             |                               |                               |             | 0.239*|
| Male                                            | 30 (57.7)                     | 24 (46.2)                     | 54 (51.9)   |       |
| Female                                          | 22 (42.3)                     | 28 (53.8)                     | 50 (48.1)   |       |
| Gestational Age                                 |                               |                               |             | 0.184*|
| Extremely Preterm (<28 weeks)                   | 5 (9.6)                       | 3 (5.8)                       | 8 (7.7)     |       |
| Very Preterm (28 - <32 weeks)                   | 8 (15.4)                      | 18 (34.6)                     | 26 (25)     |       |
| Moderate Late Preterm (32 - <37 weeks)          | 33 (63.5)                     | 24 (46.2)                     | 57 (54.8)   |       |
| Term (≥37 weeks)                                | 6 (11.5)                      | 7 (13.5)                      | 13 (12.5)   |       |
| Birth Weight                                    |                               |                               |             | 0.008*|
| Normal                                          | 3 (5.8)                       | 7 (13.5)                      | 10 (9.6)    |       |
| Low Birth Weight (<2500 grams)                  | 27 (51.9)                     | 16 (30.8)                     | 43 (41.3)   |       |
| Very Low Birth Weight (<1500 grams)             | 20 (38.5)                     | 17 (32.7)                     | 37 (35.6)   |       |
| Extremely Low Birth Weight (<1000 grams)        | 2 (3.8)                       | 12 (23.1)                     | 14 (13.5)   |       |
| Lubschenco score                                |     |                               |             | 0.512*|
| Appropriate for Gestational Age (AGA)            | 39 (75)                       | 36 (69.2)                     | 75 (72.1)   |       |
| Small for Gestational Age (SGA)                 | 13 (25)                       | 16 (30.8)                     | 29 (27.9)   |       |
| Mode of delivery                                |                               |                               |             | 0.222*|
| Spontaneous                                     | 16 (30.7)                     | 22 (42.3)                     | 38 (36.5)   |       |
| Sectio Caesaria                                  | 35 (67.3)                     | 28 (53.8)                     | 63 (60.6)   |       |
| Others                                          | 1 (2)                         | 2 (3.9)                       | 3 (2.9)     |       |
| History of premature rupture of membrane        |                               |                               |             | 1.000*|
| Yes                                             | 15 (28.8)                     | 15 (28.8)                     | 30 (28.8)   |       |
| No                                              | 37 (71.2)                     | 37 (71.2)                     | 74 (71.2)   |       |
| History of Preeclampsia/Eclampsia               |                               |                               |             | 0.543*|
| Yes                                             | 21 (40.4)                     | 18 (34.6)                     | 39 (37.5)   |       |
| No                                              | 31 (59.6)                     | 34 (65.4)                     | 65 (62.5)   |       |
| History of Prenatal Steroid                     |                               |                               |             | 0.464*|
| Yes                                             | 12 (23.1)                     | 9 (17.3)                      | 21 (20.2)   |       |
| No                                              | 40 (76.9)                     | 43 (82.7)                     | 83 (79.8)   |       |
| Onset Neonatal Sepsis                           |                               |                               |             | 0.003*|
| Early-onset Neonatal Sepsis (EOS)               | 13 (25)                       | 28 (53.8)                     | 41 (39.4)   |       |
| Late-onset Neonatal Sepsis (LOS)                | 39 (75)                       | 24 (46.2)                     | 63 (60.6)   |       |
| Leucocyte count (median (IQR))                  | 11700 (6405 – 17590)          | 10215 (4920 – 16100)          | 11265 (5745 – 17265) | 0.465** |
| Neutrophil count (ANC) (median (IQR))           | 7310 (4920 – 11580)           | 5390 (2995 – 10495)           | 6375 (3260 – 11205) | 0.155** |
| Lymphocyte count (median (IQR))                 | 1840 (1120 – 3145)            | 2155 (1270 – 3540)            | 2010 (1160 – 3245) | 0.359** |

* Chi-Square’s test

** Mann-Whitney U test
Figure 2. *Box plot* neutrophil-to-lymphocyte ratio (NLR) and CRP based on blood culture (Mann-Whitney U test)

The ratio of neutrophil-to-lymphocyte ratio (NLR) as a marker of neonatal sepsis took place the area under the curve (AUC) of 0.63 (95% CI 0.528-0.741) with a cut-off
2.12. The AUC of NLR did not differ statistically when compared to CRP at a cutoff 2.7 mg/dL which was 0.69 (95% CI 0.592-0.793; p=0.454) (figure 3a). A better AUC of 0.725 (95% CI 0.636 - 0.814) can be achieved if NLR is combined with CRP (figure 3b). The NLR have high sensitivity of 80.77%, but a low specificity of 42.31%. Positive predictive value (PPV) and negative predictive value (NPV) were 58.33% and 68.75% with an accuracy of 62.5 %. The combination of NLR and CRP have 75% sensitivity, 67.3% specificity, 69.64% PPV, 72.9% NPV and 71.15% accuracy. Neonates with an NLR ≥2.12 have almost twice the risk of giving a positive blood culture result (RR 1.867 95% CI 1.077-3.235; p = 0.011) whereas when combined with a CRP > 2.7 mg/dL, the value of the risk is more than doubled to give results positive blood culture (RR 2.106, 95% CI 1.396-3.179, p=0.000).

Figure 3. Area under curve NLR and CRP (a) and NLR combined with CRP as initial marker of neonatal sepsis
Discussion

Preterm and LBW infants were the highest subjects to have neonatal sepsis in this study. This results was similar with the previous study reported in Denpasar in 2008 (50% preterm and 53.6% LBW neonates) and in Surabaya in 2010 (77.78% preterm and 32% LBW neonates).\textsuperscript{8,12} The incidence of neonatal sepsis is inversely related to birth weight and gestational age. It is mostly found in infants with very low birth weight/ VLBW (<1500 g) and GA <28 weeks.\textsuperscript{5,13} Prematurity with sepsis as complication was the leading cause of neonatal mortality. Higher neonatal death also found at lower gestational with majority born at GA <26. The majority of neonatal death were noted in less than 1000
grams of birth weight too. More male sex was found in this study by 51.9%. Similar results were reported by several previous studies ranging from 55.2 to 59.9% although there was no difference in the risk of neonatal sepsis by sex.15–19

As many as 75% of positive blood cultures were obtained in LOS and dominated by gram-negative bacteria. It is significantly 3.5 times more common than EOS. Late-onset neonatal sepsis (LOS) with positive blood cultures was also reported in previous studies by Shehab el-Din at 65% and Yusef at 72%.16,20 Late-onset neonatal sepsis (LOS) was associated with nosocomial bacteria and more common in preterm neonates due to long term treatment in NICU.1,21,22 Immature immune responses and high invasive life support such as central venous catheters (CVC) or endotracheal tubes and total parenteral nutrition (TPN) support make preterm neonates more susceptible to infection than term neonates.1,5,21 Dominated of gram-negative bacteria was also reported in previous study and it was found that intrapartum antibiotic, TPN duration, CVC duration and mechanical ventilation duration were potential maternal and neonatal risk factor for late-onset gram negative sepsis (LOGNS).19 Nevertheless, the use of CVC, endotracheal tubes and TPN were not evaluated in this study. Low humoral immune system in preterm neonates occurs because the transfer of IgG transplacental from the mother to the fetus begins in the second trimester and reaches a peak in the third trimester of pregnancy.21,22

Nearly 90% of neonatal sepsis with positive blood cultures in this study had normal leukocyte counts (4000-34000/mm³). Abnormal total leukocytes count are only found in 8 neonates (4 EOS and 4 LOS) with total leukocyte counts <4000/mm³ and no neonates with total leukocyte counts >34000/mm³. Sucilathamang also reported that normal total leukocyte counts were obtained in 12/14 (85.72%) neonates with positive blood culture results.23 Normal total leukocyte count cannot rule out the presence of neonatal sepsis because 50% of neonatal sepsis with positive blood cultures having a normal total leukocyte count.9

Higher median NLR was found in proven neonatal sepsis or neonates with positive blood cultures. Higher mean NLR in positive blood cultures also reported by Ozdemir with 3.69 ± 3.0 compared to 1.56 ± 1.83 (p <0.001) in negative blood cultures.1 Significantly higher NLR compared to healthy neonates were also reported in several previous studies. The term neonatal sepsis group had a significantly higher NLR than the healthy term neonate reported by Can with 2.88 ± 0.16 and 0.21 ± 0.12, p = 0.02 and by
Overall studies in the neonatal sepsis group have higher NLR values compared to normal NLR values that were previously reported by Hamiel in the healthy neonatal or pediatric population with an average NLR 0.52-0.91. The high NLR value in the sepsis group is due to an imbalance between the levels of neutrophils and lymphocytes. Increasing neutrophils is a first-line defense mechanism in the primary role of neutrophils as an innate immune system to fight bacterial infections and will stimulate the process of emergency granulopoiesis. An increase in neutrophil counts will be accompanied by a decrease in lymphopoiesis and monocytopenia and also a decrease in neutrophil apoptosis but an increase in lymphocyte apoptosis resulting in neutrophilia and lymphocytopenia.

The NLR cutoff in this study was higher than in previous studies which NLR 1.81 cutoff value in neonates with risk factors for sepsis and neonatal sepsis in Dr Moewardi Hospital Surakarta has 86.1% sensitivity, 85.1% specificity, 68.9% PPV and 94.1% NPV. The NLR cutoff in this study was lower than in some previous studies but with higher sensitivity. Omran obtained an average NLR value in 35 term neonates with positive and negative blood cultures compared to healthy term neonates of 2.9 ± 1.7 with NLR 2.7 has 80% sensitivity and 57.1% specificity with AUC of 0.791 ± 0.057. Ruslie obtained an NLR 9.4 cutoff value having a sensitivity of 61.5% and a specificity of 66.7% in 94 neonates with clinical manifestations of sepsis (positive blood culture versus negative blood culture), which was dominated by term neonates as study samples.

Area under curve (AUC) of NLR and CRP are not statistically significant different but the best AUC will be obtained if both of them are combined. Only one previous study that also reported the best AUC of 0.79 (95% CI 0.70-0.88) was found in a combination of NLR and CRP compared to NLR or CRP alone. The study was conducted by Hamiel et al. in term neonates aged 7-28 days old until 90 days old who experience serious bacterial infection (SBI) in 2012 - 2014.

This is the first study in Surabaya, Indonesia that assesses NLR as a neonatal sepsis marker. The use of NLR is expected to reduce the use of antibiotics that are not appropriate indications, complications and neonatal mortality due to infections, especially bacterial infections. A limitation of this study is that it was collected at only one research center even the transferred baby to the same setting was not included. This study also did
not include healthy neonates as a control group so that the normal range value of NLR could not be known.

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Author Contributions
KRS, MTU and ADWW developed the research design, analysis and revised the manuscript. KRS was responsible for data collection and analysis.

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No funding was received for this study.

Conflicts of Interest
None

Ethics
Ethical clearance in this study was approved by Ethical Committee in Health Research of Dr. Soetomo General Academic Surabaya (ref. no. 1047/KEPK/III/2019).

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