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Manuscript title: Neutrophil-to-Lymphocyte Ratio as an Alternative Marker of Neonatal Sepsis in Developing Countries
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Please find the attached manuscript with comments/queries from our medical editor. Please use **track changes** while revising your paper (see attached) and kindly reply to each editor's comments. We will then update the paper and once ready, we will send you a copy of the final draft in journal format for final proofread.

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Title : **NEUTROPHIL-TO-LYMPHOCYTE RATIO AS AN ALTERNATIVE MARKER OF NEONATAL SEPSIS IN DEVELOPING COUNTRIES**

Author : Khadijah Rizky Sumitro, Martono Tri Utomo, Agung Dwi Wahyu Widodo

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1	The manuscript is addressed the critical comments as seen in updated version, but still The setting name is need to be masked, (Except in page 3- Ethical consideration)	**AUTHOR REPLY** I was very grateful and appreciated for the comment that has been given. I have deleted all the setting names that have been written in the manuscript (except in page 3) and replaced them in accordance with the comments that I included in the manuscript.	1 - 2
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Oman Medical Journal (2021) Vol. 36, No. 1: -
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Neutrophil-to-Lymphocyte Ratio as an Alternative Marker of Neonatal Sepsis in Developing Countries

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ABSTRACT

Objectives: We sought to analyze the neutrophil-to-lymphocyte ratio (NLR) as an alternative marker of neonatal sepsis. **Methods:** In this cross-sectional study we undertook consecutive sampling in all inborn neonates and admitted in the Neonatal Intensive Care Unit (NICU) with clinical manifestations of neonatal sepsis. 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. A cut-off value of NLR is obtained using a receiver operating characteristic curve (ROC curve). **Results:** The median NLR value of 104 neonates who met the inclusion and exclusion criteria was 3.63 (2.39–6.12). NLR 2.12 have the area under the curve (AUC) of 0.630 (95% CI 0.528–0.741) and 0.725 (95% CI 0.636–0.814) when combined with CRP 2.70 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, calculated from 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:

Infant, Newborn; Neonatal Sepsis; Neutrophils; Lymphocytes; Intensive Care Units, Neonatal; C-Reactive Protein.

Introduction

Neonatal sepsis is a major problem for neonates around the world and contributes significant morbidity and mortality neonates (term and preterm), especially in developing countries.[1,2] It is a clinical syndrome characterized by systemic infections and the isolation of pathogens in the blood (bacteremia) and occurs in infants in the first month of life.[3–6] As many as 1.6 million neonates die each year due to infection and 60% occurred in developing countries.[7] 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).[5,8,9]

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.[10] Blood culture as a gold standard markers, took a long time and often gave negative results.[1] The neutrophil-lymphocyte ratio (NLR) ratio was an

inexpensive and part of a complete blood count that does not require additional examination.[10] 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.[11] To date, no studies in neonatal have been conducted in our hospital before so the purpose of this study was to analyze the NLR as an initial marker of neonatal sepsis that can be used in limited resource areas.

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Methods

We conducted a six month (April to September 2019) observational analytic study using a cross-sectional design with consecutive sampling in all inborn neonates and treated in NICU with clinically neonatal sepsis. Complete blood count, 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. 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 ºC or < 36 ºC), capillary refill time (CRT) > 3 seconds, abnormal leukocyte count (< 4000 or > 34000/mm^3), CRP > 10 mg/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 one day old and < 65 mmHg for ≤ 1 month old); and inflammatory variables (leukocytosis > 34 000/mm^3; leukopenia < 4000/mm^3; immature neutrophils > 10%; I/T (immature to total neutrophil) ratio > 0.2; thrombocytopenia < 100 000/mm^3; 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.[7] 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.

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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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 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 two 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 the 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 a *p*-value < 0.050. The NLR cut-off 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 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 (50.0%) neonates categorized as proven neonatal sepsis. Gram-negative bacteria dominate by 75.0% 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 NLR (p = 0.018) and CRP (p = 0.001) in the proven neonatal sepsis was significantly higher than in the suspected group [Figure 2].

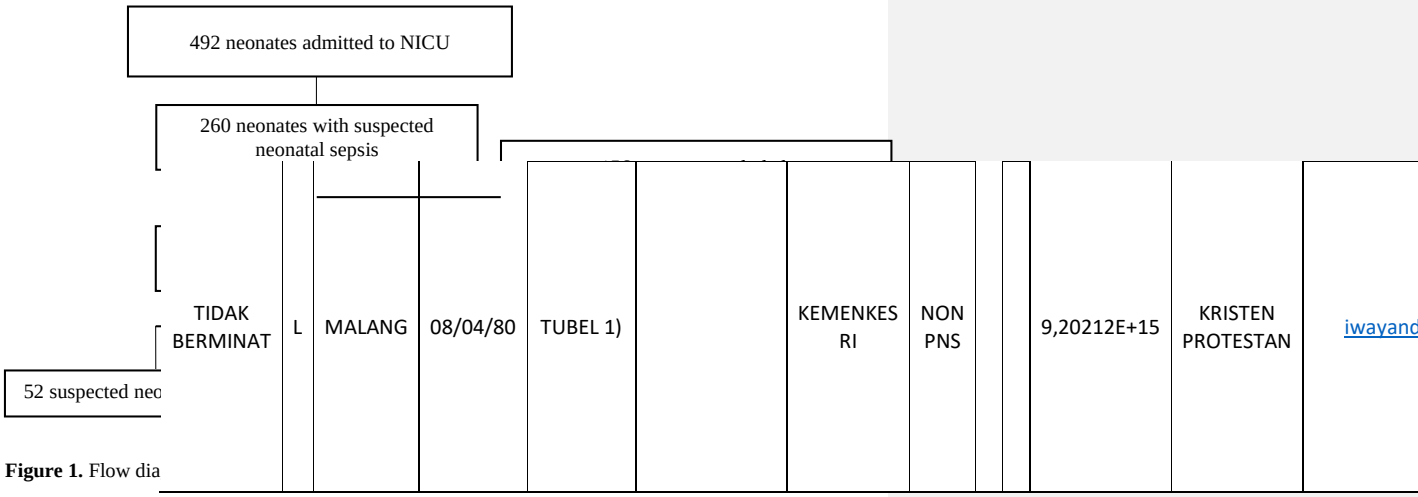


Figure 1. Flow dia

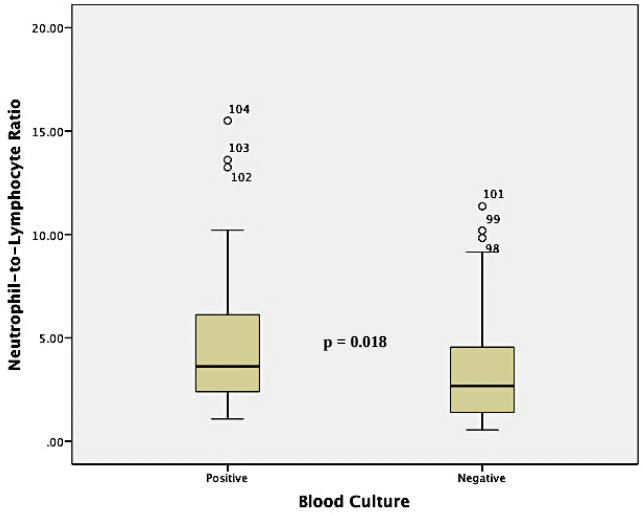
Table 1. Subjects characteristics.

	Proven Neonatal Sepsis n (%)	Suspected Neonatal Sepsis n (%)	Total n (%)	p
Sex	30 (57.7)	24 (46.2)	54 (51.9)	0.239*
Male	22 (42.3)	28 (53.8)	50 (48.1)	
Female				
Gestational age	5 (9.6)	3 (5.8)	8 (7.7)	0.184*
Extremely preterm	8 (15.4)	18 (34.6)	26 (25.0)	
(< 28 weeks)	33 (63.5)	24 (46.2)	57 (54.8)	
Very preterm	6 (11.5)	7 (13.5)	13 (12.5)	
(28 - < 32 weeks)				
Moderate late preterm				
(32 - < 37 weeks)				
Term (≥ 37 weeks)				
Birth weight	3 (5.8)	7 (13.5)	10 (9.6)	0.008*
Normal	27 (51.9)	16 (30.8)	43 (41.3)	
Low birth weight	20 (38.5)	17 (32.7)	37 (35.6)	
(< 2500 grams)	2 (3.8)	12 (23.1)	14 (13.5)	
Very low birth weight				
(< 1500 grams)				
Extremely low birth weight (< 1000 grams)				
Lubschenco score	39 (75.0)	36 (69.2)	75 (72.1)	0.512*
Appropriate for gestational age	13 (25.0)	16 (30.8)	29 (27.9)	
Small for				

gestational age				
Mode of delivery	16 (30.8)	22 (42.3)	38 (36.5)	0.222*
Spontaneous	35 (67.3)	28 (53.8)	63 (60.6)	
Sectio caesaria	1 (1.9)	2 (3.8)	3 (2.9)	
Others				
History of premature rupture of membrane	15 (28.8)	15 (28.8)	30 (28.8)	1.000*
Yes	37 (71.2)	37 (71.2)	74 (71.2)	
No				
History of preeclampsia/ eclampsia	21 (40.4)	18 (34.6)	39 (37.5)	0.543*
Yes	31 (59.6)	34 (65.4)	65 (62.5)	
No				
History of prenatal steroid	12 (23.1)	9 (17.3)	21 (20.2)	0.464*
Yes	40 (76.9)	43 (82.7)	83 (79.8)	
No				
Onset neonatal sepsis	13 (25.0)	28 (53.8)	41 (39.4)	0.003*
Early-onset neonatal sepsis	39 (75.0)	24 (46.2)	63 (60.6)	
Late-onset neonatal sepsis				
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**p**
Total leucocyte count, /mm^3	11700 (6405 – 17590)	10215 (4920 – 16010)	11265 (5745 – 17265)	0.465**
Absolute neutrophil count, /mm^3	7310 (4920 – 11580)	5390 (2995 – 10495)	6375 (3260 – 11205)	0.155**
Absolute lymphocyte count, /mm^3	1840 (1120 – 3145)	2155 (1270 – 3540)	2010 (1160 – 3245)	0.359**

∗ Chi-Square'*stest*

** *Mann-Whitney U test*



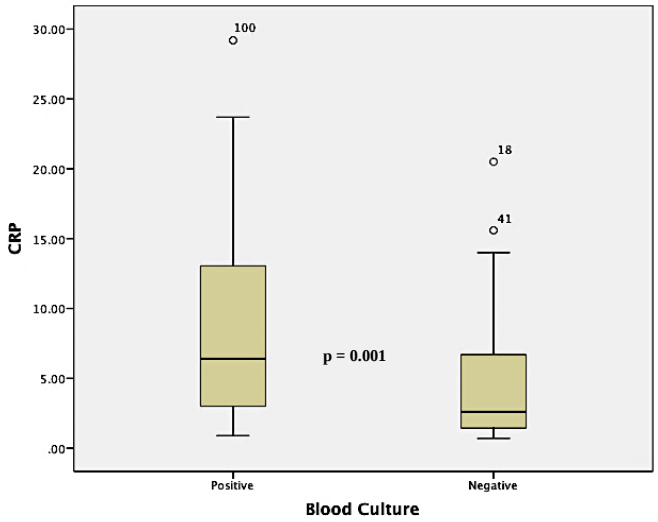


Figure 2. Box plot of neutrophil-to-lymphocyte ratio (NLR) and CRP based on blood culture (Mann-Whitney U test)

The ratio of NLR as a marker of neonatal sepsis took place the AUC of 0.630 (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 cut-off 2.70 mg/dL which was 0.690 (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 80.8%, but a low specificity of 42.3%. Positive predictive value (PPV) and negative predictive value (NPV) were 58.3% and 68.8% with an accuracy of 62.5%. The combination of NLR and CRP have 75.0% sensitivity, 67.3% specificity, 69.6% PPV, 72.9% NPV and 71.2% accuracy. Neonates with an NLR $\geq$ 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 > 2.70 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, $p < 0.001$).

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

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It should be written "Area under curve of NLR and CRP **(a)** and NLR combined with CRP **(b)** as initial marker of neonatal sepsis."

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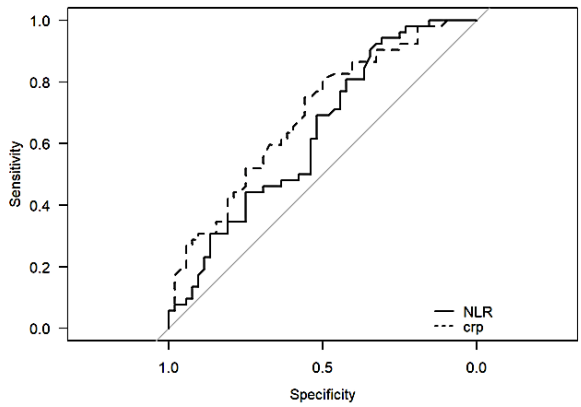
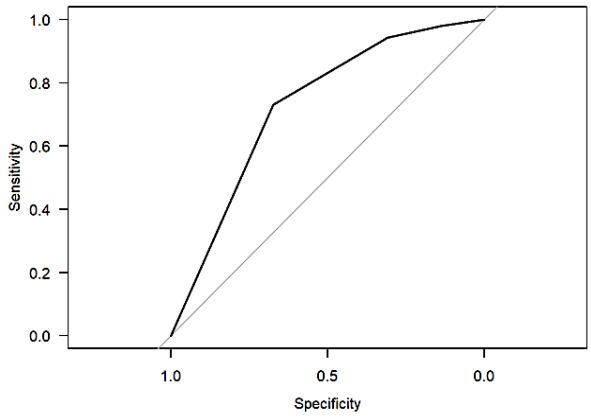


(a)



(b)

Discussion

Preterm and low birth weight (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).[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.[5,13] Prematurity with sepsis as complication was the leading cause of neonatal mortality. Higher neonatal death also found at lower gestational age with majority born at < 26 weeks. The majority of neonatal death were noted in infants with a birth weight of less than 1000 grams.[14] 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 75.0% of positive blood cultures were obtained in late-onset neonatal sepsis (LOS) and dominated by gram-negative bacteria. It is significantly 3.5-times more common than early-onset neonatal sepsis (EOS). LOS with positive blood cultures was also reported in previous studies by Shehab el-Din at 65% and Yusef at 72%.[16,20] LOS was associated with nosocomial bacteria and more common in

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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.[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/mm^3). Abnormal total leukocytes count are only found in eight neonates (four EOS and four LOS) with total leukocyte counts < 4000/mm^3 and no neonates with total leukocyte counts > 34000/mm^3. Sucilathangam 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 50.0% 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 Omran with 2.9±1.7 and 1.6±0.4, $p < 0.001$.[24,25] 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.[11] 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 monocytopoiesis and also a decrease in neutrophil apoptosis but an increase in lymphocyte apoptosis resulting in neutrophilia and lymphocytopenia.[1,26–28]

The NLR cut-off in this study was higher than in previous studies which NLR 1.81 cut-off 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.[29] The NLR cut-off 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.[25] Ruslie obtained an NLR 9.4 cut-off 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.[30]

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 in 2012–2014.[11]

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.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

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The authors thank all patients who have been involved in this study, ~~to Dr. Soetomo General Academic in Surabaya~~ to Ethical Committee in Health Research of Dr. Soetomo General Academic Surabaya for giving permission and ethical clearance (ref. no. 1047/KEPK/III/2019) so that this study can proceed, and all team members and colleagues for assisting this research.

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References

1. Alkan Ozdemir S, Arun Ozer E, Ilhan O, Sutcuoglu S. Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? J Clin Lab Anal 2018 May;32(4):e22338 .

2. Hasibuan BS. Comparison of microbial pattern in early and late onset neonatal sepsis in referral center Haji Adam Malik hospital Medan Indonesia. IOP Conf Ser Earth Enviromental Sci 2018;125(1):012053.

3. Edwards MS, Baker CJ. Sepsis in the newborn. In: Gershon A, Hotez P, Katz S, editors. Krugman's Infectious Disease of Children. 11th ed. Philadelphia, 2003. p. 545–563.

4. Polin RA; Committee on Fetus and Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics 2012 May;129(5):1006-1015 .

5. Bany-Mohammed F. Sepsis. In: Gomella TL, Cunningham MD, Eyal FG, editors. Neonatology : management, procedures, on-call problems, disesase and drugs. 7th ed. New York, 2013. p. 865–874.

6. Coetzee M, Mbowane N, De Witt T. Neonatal sepsis: Highlighting the principles of diagnosis and management. S Afr J Child Heal 2017 Jul;11(2):99-103 .

7. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med 2005 May;6(3)(Suppl):S45-S49 .

8. Kardana IM. Incidence and factors associated with mortality of neonatal sepsis. Paediatr Indones 2011 May;51(3):144-148 .

9. Gomella TL. Postdelivery antibiotic. In: Gomella TL, Cunningham MD, Eyal FG, editors. Neonatology : management, procedures, on-call problems, disesase and drugs. 7th ed. New York, 2013. p. 492–501.

10. Holub M, Beran O, Kaspříková N, Chalupa P. Neutrophil to lymphocyte count ratio as a biomarker of bacterial infections. Cent Eur J Med 2012 Jan;7(2):258-261.

11. Hamiel U, Bahat H, Kozer E, Hamiel Y, Ziv-Baran T, Goldman M. Diagnostic markers of acute infections in infants aged 1 week to 3 months: a retrospective cohort study. BMJ Open 2018 Jan;8(1):e018092 .

12. Utomo MT. Neonatal sepsis in low birth weight infants in Dr. Soetomo General Hospital. Indones J Trop Infect Dis 2010 May-Aug;1(2):86-89 .

13. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. Arch Dis Child Fetal Neonatal Ed 2015 May;100(3):F257-F263 .

14. Abdellatif M, Ahmed M, Bataclan MF, Khan AA, Al Battashi A, Al Maniri A. The patterns and causes of neonatal mortality at a tertiary hospital in oman. Oman Med J 2013 Nov;28(6):422-426.

15. Ozkan H, Cetinkaya M, Koksal N, Celebi S, Hacımustafaoglu M. Culture-proven neonatal sepsis in preterm infants in a neonatal intensive care unit over a 7 year period: coagulase-negative Staphylococcus as the predominant pathogen. Pediatr Int 2014 Feb;56(1):60-66 .

16. Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of neonatal sepsis and implicated pathogens: a study from Egypt. Biomed Res Int 2015;2015(1):509484 .

17. Mahallei M, Rezaee MA, Mehramuz B, Beheshtirooy S, Abdinia B. Clinical symptoms, laboratory, and microbial patterns of suspected neonatal sepsis cases in a children's referral hospital in northwestern Iran. Medicine (Baltimore) 2018 Jun;97(25):e10630 .

18. Murthy S, Godinho MA, Guddattu V, Lewis LE, Nair NS. Risk factors of neonatal sepsis in India: A systematic review and meta-analysis. PLoS One 2019 Apr;14(4):e0215683 .

19. Abdellatif M, Al-Khabori M, Rahman AU, Khan AA, Al-Farsi A, Ali K. Outcome of late-onset neonatal sepsis at a tertiary hospital in Oman. Oman Med J 2019 Jul;34(4):302-307 .

20. Yusef D, Shalakhti T, Awad S, Algharaibeh H, Khasawneh W. Clinical characteristics and epidemiology of sepsis in the neonatal intensive care unit in the era of multi-drug resistant organisms: A retrospective review. Pediatr Neonatol 2018 Feb;59(1):35-41 .

21. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004 Jul;17(3):638-680 .

22. Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr 2015 Feb;61(1):1-13 .

23. Sucilathangam G, Amuthavalli K, Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palaniappan N. Early diagnostic markers for neonatal sepsis: comparing procalcitonin (pct) and c-reactive protein (crp). J Clin Diagn Res 2012 May;6(4):627-631.

24. Can E, Hamilcikan Ş, Can C. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. J Pediatr Hematol Oncol 2018 May;40(4):e229-e232 .

25. Omran A, Maaroof A, Saleh MH, Abdelwahab A. Salivary C-reactive protein, mean platelet volume and neutrophil lymphocyte ratio as diagnostic markers for neonatal sepsis. J Pediatr (Rio J) 2018 Jan - Feb;94(1):82-87 .

26. Wesche DE, Lomas-Neira JL, Perl M, Chung CS, Ayala A. Leukocyte apoptosis and its significance in sepsis and shock. J Leukoc Biol 2005 Aug;78(2):325-337 .

27. Manz MG, Boettcher S. Emergency granulopoiesis. Nat Rev Immunol 2014 May;14(5):302-314 .

28. Hwang SY, Shin TG, Jo IJ, Jeon K, Suh GY, Lee TR, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. Am J Emerg Med 2017 Feb;35(2):234-239 .

29. Nugroho ID. Nilai diagnostik neutrophil to lymphocyte count ratio sebagai penanda awal pada neonatus terinfeksi [Thesis]. Universitas Sebelas Maret; 2018. Available from: https://eprints.uns.ac.id/40470/.

30. Ruslie RH, Tjipta DG, Samosir CT, Hasibuan BS. Bacterial pattern and role of laboratory parameters as marker for neonatal sepsis. IOP Conf Ser Earth Environ Sci 2018;125(1):012057.

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Journal OMSB <omj@omsb.org> Thu, Dec 17, 2020 at 6:10 PM
To: Martono Utomo <mrmartono73@gmail.com>

Manuscript title: Neutrophil-to-Lymphocyte Ratio as an Alternative Marker of Neonatal Sepsis in Developing Countries
Manuscript No.: OMJ-D-20-00025

Dear Dr. Martono Tri Utomo,

Good day! I hope everything is well.
Please find attached additional query from our medical editor.
We hope to receive your response soon.

Thank you.
Best regards,
Charie

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On Mon, Dec 14, 2020 at 7:34 AM Martono Utomo <mrmartono73@gmail.com> wrote:

> Dear Charie Ricafort,
>
> Hope you are doing well.
> Thank you for the correction and suggestion,
> I already update the paper as attached.
>
> Can't wait to see the final draft soon.
>
> Regards,
> Martono Tri Utomo

On Sun, Dec 13, 2020 at 11:48 AM Journal OMSB <omj@omsb.org> wrote:

> **Manuscript title: Neutrophil-to-Lymphocyte Ratio as an Alternative Marker of Neonatal Sepsis in Developing Countries**
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> Dear Dr. Martono Tri Utomo,
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> Good day! I hope everything is well.
> Please find the attached manuscript with comments/queries from our medical editor. Please use **track changes** while revising your paper (see attached) and kindly reply to each editor's comments. We will then

update the paper and once ready, we will send you a copy of the final draft in journal format for final proofread.

We hope to receive your response no later than **15 December 2020.**
Thank you very much in advance for your cooperation.

Regards,
Charie Ricafort

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رؤيتنا: الإرتقاء بالمهن الطبية لرعاية صحية متميزة

رسالتنا: المجلس العماني للاختصاصات الطبية مؤسسة حكومية مستقلة، تنمي الكوادر الصحية من خلال تأهيل الأطباء المتخصصين، وضمان كفاءة العاملين في مجال الرعاية الصحية لمجتمع أكثر صحة وسعادة

Our Vision: Leading the advancement of medical professions to ensure excellence in healthcare

Our Mission: We are an autonomous body furthering the growth of human resources for health, through developing specialized physicians and assuring the competencies of health care professionals for a healthier and a happier community

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NEUTROPHIL-TO-LYMPHOCYTE RATIO AS AN ALTERNATIVE MARKER OF NEONATAL SEPSIS IN DEVELOPING COUNTRIES

ABSTRACT

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

Methods: Consecutive sampling in all inborn neonates ~~in Dr. Soetomo General Academic Surabaya~~ 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 ~~of the Children's Health Department - Dr. Soetomo General Academic Hospital~~. 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 $\geq$ 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*

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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. [1,2] 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.[3–6] As many as 1.6 million neonates died each year due to infection and 60% of them occurred in developing countries.[7] 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). [5,8,9]

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.[10] Blood culture as a gold standard markers, took a long time and often gave negative results.[1] The neutrophil-lymphocyte ratio (NLR) ratio was an inexpensive and part of a complete blood count that does not require additional examination.[10] 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.[11] Until now, no studies has been conducted in our hospital ~~at Dr. Soetomo General Academic Surabaya~~ 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.

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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 ~~from the Children's Health Department - Dr. Hospital. Soetomo~~. 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^{o}C or <36^{o}C), capillary refill time (CRT) >3 seconds, abnormal leukocyte count (<4000 or >34000/mm^{3}), CRP

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>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/mm^3; leukopenia <4,000/mm^3; immature neutrophils >10%; I/T (immature to total neutrophil) ratio >0.2; thrombocytopenia <100,000/mm^3; 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.[7] 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 ~~Dr. Soetomo General Academic~~ 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 ~~outside Dr. Soetomo General Academic Surabaya~~) 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).

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).

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 ~~Sanglah Hospital~~ Denpasar in 2008 (50% preterm and 53.6% LBW neonates) and in ~~Dr Soetomo General~~

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~~Academic~~ Surabaya in 2010 (77.78% preterm and 32% LBW neonates).[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.[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.[14] 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/mm^3). Abnormal total leukocytes count are only found in 8 neonates (4 EOS and 4 LOS) with total leukocyte counts <4000/mm^3 and no neonates with total leukocyte counts >34000/mm^3. Sucilathangam 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]

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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 Omran with 2.9 ± 1.7 and 1.6 ± 0.4, $p < 0.001$. [24,25] 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. [11] 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 monocytopoiesis and also a decrease in neutrophil apoptosis but an increase in lymphocyte apoptosis resulting in neutrophilia and lymphocytopenia. [1,26–28]

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.[29] 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. [25] 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. [30]

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.[11]

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.

Acknowledgements

The authors thank all patients who have been involved in this study, to Dr. Soetomo General Academic in Surabaya for giving permission so that this study can proceed, and all team members and colleagues for assisting this research.

Author Contributions

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

Funding

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).

References

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1. Ozdemir SA, Ozer EA, Ilhan O, Sutcuoglu S. Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? J Clin Lab Anal 2018 May;32(4):e22338.
2. Hasibuan BS. Comparison of microbial pattern in early and late onset neonatal

sepsis in referral center Haji Adam Malik hospital Medan Indonesia. IOP Conf Ser Earth Enviromental Sci 2018;125(1):012053.

3. Edwards MS, Baker CJ. Sepsis in the newborn. In: Gershon A, Hotez P, Katz S, editors. Krugman's Infectious Disease of Children. 11th ed. Philadelphia, 2003. p. 545–563.
4. Polin RA. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics 2012 May;129(5):1006–1015.
5. Bany-Mohammed F. Sepsis. In: Gomella TL, Cunningham MD, Eyal FG, editors. Neonatology : management, procedures, on-call problems, disesase and drugs. 7th ed. New York, 2013. p. 865–874.
6. Coetzee M, Mbowane N, De Witt T. Neonatal sepsis: Highlighting the principles of diagnosis and management. S Afr J Child Heal 2017 Jul;11(2):99–103.
7. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med 2005 May;6(3):S45–49.
8. Kardana IM. Incidence and factors associated with mortality of neonatal sepsis. Paediatr Indones 2011 May;51(3):144–148.
9. Gomella TL. Postdelivery antibiotic. In: Gomella TL, Cunningham MD, Eyal FG, editors. Neonatology : management, procedures, on-call problems, disesase and drugs. 7th ed. New York, 2013. p. 492–501.
10. Holub M, Beran O, Kaspříková N, Chalupa P. Neutrophil to lymphocyte count ratio as a biomarker of bacterial infections. Cen Eur J Med 2012 Jan;7(2):258–261.
11. Hamiel U, Bahat H, Kozer E, Hamiel Y, Ziv-Baran T, Goldman M. Diagnostic markers of acute infections in infants aged 1 week to 3 months: a retrospective cohort study. BMJ Open 2018;8(1):e018092.
12. Utomo MT. Neonatal sepsis in low birth weight infants in Dr . Soetomo General Hospital. Indones J Trop Infect Dis 2010 May-Aug;1(2):86–89.
13. Dong Y, Speer CP. Late-onset neonatal sepsis:Recent developments. Arch Dis Child Fetal Neonatal Ed 2015 May;100(3):F257-263.
14. Abdellatif M, Ahmed M, Bataclan MF, Khan AA, Al Battashi A, Al Maniri A. The patterns and causes of neonatal mortality at a tertiary hospital in Oman. Oman Med J 2013 Nov;2(6)8:422–426.
15. Ozkan H, Cetinkaya M, Koksal N, Celebi S, Hacimustafaoglu M. Culture-proven

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16. Shehab El-Din EMR, El-Sokkary MMA, Bassiouny MR, Hassan R. Epidemiology of neonatal sepsis and implicated pathogens: a study from Egypt. Biomed Res Int 2015 Jun;2015(1):1–11.
17. Mahallei M, Rezaee MA, Mehramuz B, Beheshtirooy S, Abdinia B. Clinical symptoms, laboratory, and microbial patterns of suspected neonatal sepsis cases in a children's referral hospital in northwestern Iran. Medicine (Baltimore) 2018 Jun;97(25):e10603.
18. Murthy S, Godinho MA, Guddattu V, Lewis LES, Sreekumaran Nair N. Risk factors of neonatal sepsis in India: a systematic review and meta-analysis. PLoS One 2019 Apr;14(4):e0215683.
19. Abdellatif M, Al-Khabori M, Ur Rahman A, Khan AA, Al-Farsi A, Ali K. Outcome of late-onset neonatal sepsis at a tertiary hospital in Oman. Oman Med J 2019 Jul;34(4):302–307.
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21. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004 Jul;17(3):638–680.
22. Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr 2015 Feb;61(1):1–13.
23. Sucilathangam G, Amuthavalli K, Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palaniappan N. Early diagnostic markers for neonatal sepsis: comparing procalcitonin (pct) and c-reactive protein (crp). J Clin Diagnostic Res 2012 May;6(4):627–631.
24. Can E, Hamilcikan Ş, Cen C. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. J Pediatr Hematol Oncol 2018 May;40(4):e229-e232.
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mean platelet volume and neutrophil lymphocyte ratio as diagnostic markers for neonatal sepsis. J Pediatr (Rio J) 2018 Jan-Feb;94(1):82–87.

26. Wesche DE. Leukocyte apoptosis and its significance in sepsis and shock. J Leukoc Biol 2005 Aug;78(2):325–337.
27. Manz MG, Boettcher S. Emergency granulopoiesis. Nat Rev Immunol 2014 May;14(5):302–314.
28. Hwang SY, Shin TG, Jo IJ, Jeon K, Suh GY, Lee TR, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. Am J Emerg Med 2017 Feb;35(2):234–239.
29. Nugroho ID. Nilai diagnostik neutrophil to lymphocyte count ratio sebagai penanda awal pada neonatus terinfeksi [Thesis]. Universitas Sebelas Maret; 2018. Available from: https://eprints.uns.ac.id/40470/.
30. Ruslie RH, Tjipta DG, Samosir CT, Hasibuan BS. Bacterial pattern and role of laboratory parameters as marker for neonatal sepsis. IOP Conf Ser Earth Environ Sci 2018;125(1):012057.

Figure 1. Flow diagram the research design

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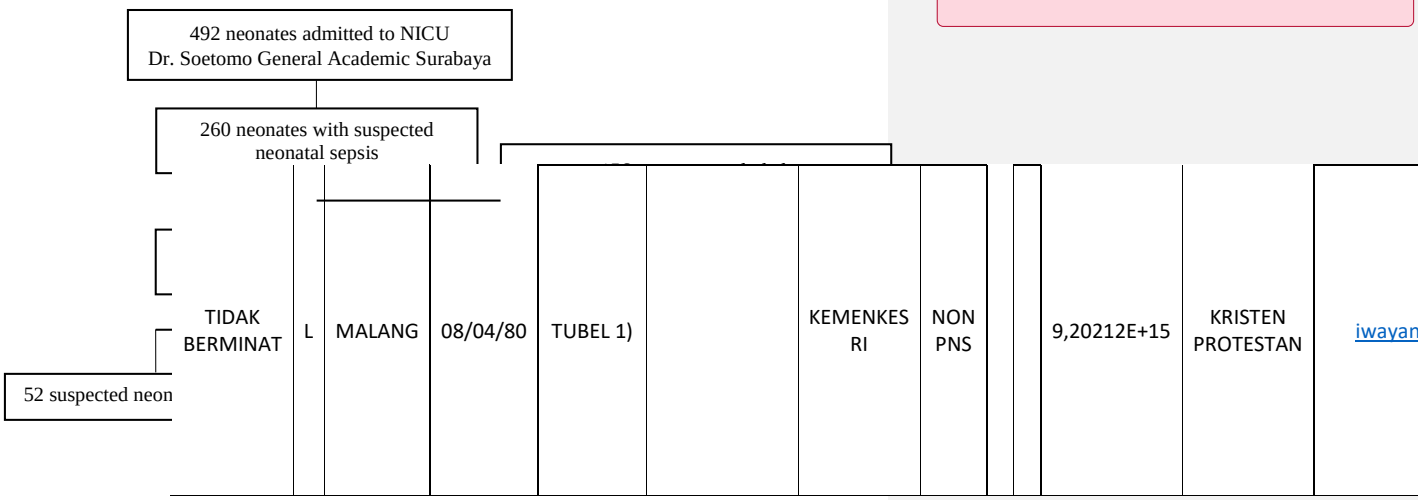


Table 1. Subjects Characteristic

	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)	
	median (IQR)	**median (IQR)**	**Median (IQR)**	**p**
Total leucocyte count (/mm^3)	11700 (6405 – 17590)	10215 (4920 – 16010)	11265 (5745 – 17265)	0.465**
Absolute neutrophil count (ANC) (/mm^3)	7310 (4920 – 11580)	5390 (2995 – 10495)	6375 (3260 – 11205)	0.155**
Absolute lymphocyte count (/mm^3)	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)

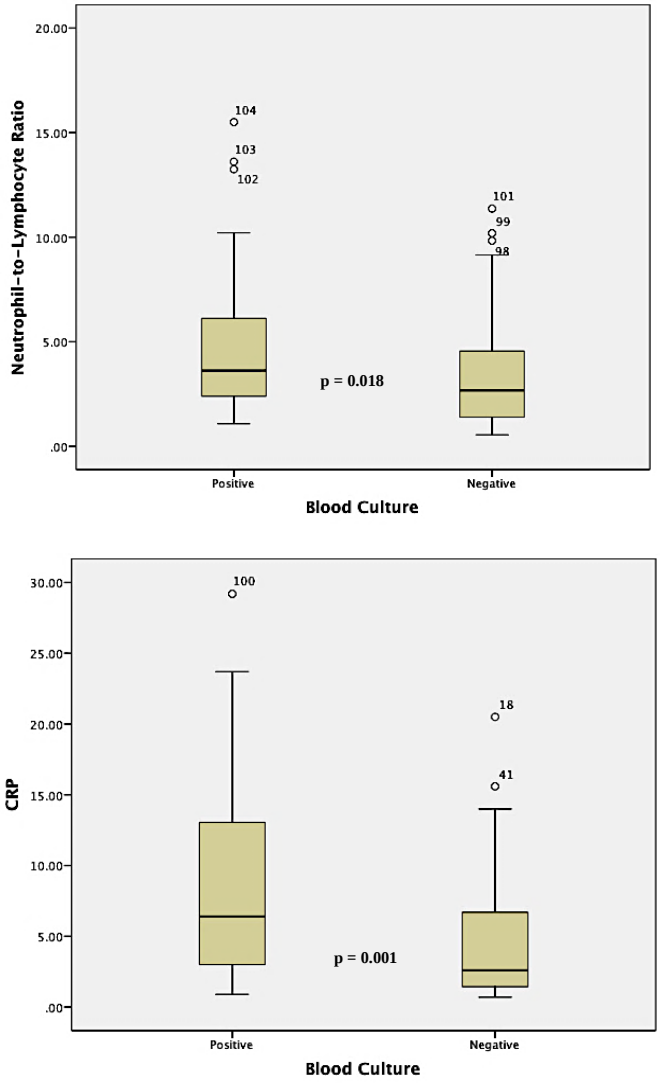
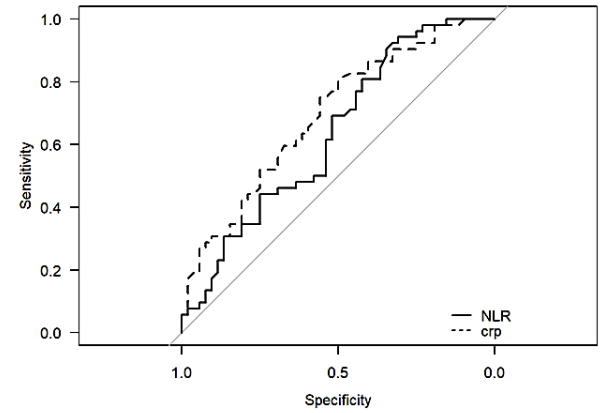
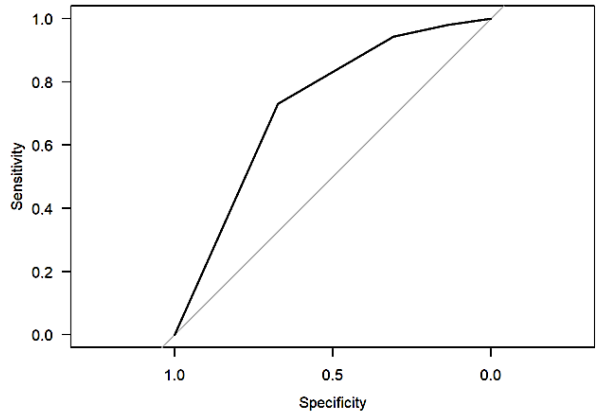


Figure 3. Area under curve NLR and CRP (a) and NLR combined with CRP as initial marker of neonatal sepsis





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ORIGINAL ARTICLE

Oman Medical Journal [2021], Vol. 36, No. 1: e214

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

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ABSTRACT

Objectives: We sought to analyze the neutrophil-to-lymphocyte ratio (NLR) as an alternative marker of neonatal sepsis. ***Methods:*** In this cross-sectional study, we undertook consecutive sampling in all inborn neonates admitted to the Neonatal Intensive Care Unit with clinical manifestations of neonatal sepsis. Neonates with congenital anomalies and referred neonates were excluded. Complete blood count, C-reactive protein (CRP), and blood culture were carried out as the septic workup examinations based on the local Clinical Practical Guidelines. NLR is obtained by dividing the absolute count of neutrophils from lymphocytes manually. A cut-off value of NLR is obtained using a receiver operating characteristic curve. ***Results:*** The median NLR value of the 104 neonates who met the inclusion and exclusion criteria was 3.63 (2.39–6.12). Neonates with NLR of 2.12 have the area under the curve of 0.630 (95% confidence interval (CI): 0.528–0.741) and 0.725 (95% CI: 0.636–0.814) when combined with CRP = 2.70 mg/dL. Neonates with NLR ≥ 2.12 in clinical neotnatal sepsis had almost double the risk of providing positive blood culture results (respiratory rate = 1.867, 95% CI: 1.077–3.235; p = 0.011). ***Conclusions:*** NLR, calculated from 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.

Neonatal sepsis is a major problem for neonates worldwide and contributes to significant morbidity and mortality (term and preterm), especially in developing countries.[1,2] It is a clinical syndrome characterized by systemic infections and the isolation of pathogens in the blood (bacteremia) and occurs in infants in the first month of life.[3–6] As many as 1.6 million neonates die each year due to infection with 60% of deaths occurring in developing countries.[7] Neonatal sepsis is reported as 1–5 per 1000 live births in developed countries, and a higher incidence is reported in developing countries (10–50 per 1000 live births).[5,8,9]

Routine parameters used for neonatal sepsis have varying diagnostic values such as total leukocyte count, absolute neutrophil count (ANC), immature/total neutrophil (I/T) ratio, and C-reactive protein (CRP). Procalcitonin, a specific bacterial infection marker, has a high price and not always available in various health service facilities, especially in rural areas.[10] Blood culture as a gold standard marker, takes a long time, and often gave negative results.[1] The neutrophil-lymphocyte ratio (NLR) is inexpensive and included as part of complete blood counts and does not require additional examination.[10] 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.[11] To date, no studies in neonates have been conducted in our hospital previously, so the purpose of this study was to analyze the NLR as an initial marker of neonatal sepsis that can be used in limited-resource areas.

METHODS

We conducted a-six-month (April to September 2019) observational analytical study using a cross-sectional design with consecutive sampling in all inborn neonates treated in Neonatal Intensive

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Care Unit (NICU) with clinically neonatal sepsis. Complete blood count, CRP, and blood culture were carried out before giving antibiotics according to the local Clinical Practice Guidelines. As mentioned by Haque, suspected neonatal sepsis was characterized by the presence of one or more fetal inflammatory response syndrome (FIRS) criteria in conjunction with signs and symptoms of infection. FIRS manifested by at least two of the following criteria, tachypnea (respiratory rate (RR)/ > 60 bpm) 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 > 34 000/mm^3), CRP > 10 mg/dL, IL-6 or IL-8 > 70 pg/mL, and positive 16S rRNA 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, and 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 one day old, and < 65 mmHg for ≤ 1 month old), and inflammatory variables (leukocytosis > 34 000/mm^3; leukopenia < 4000/mm^3; immature neutrophils > 10%; I/T ratio > 0.2; thrombocytopenia < 100 000/mm^3; 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 16S rRNA genes PCR.[7] Basic characteristics 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 steroid use, and neonatal sepsis onset were evaluated in this study.

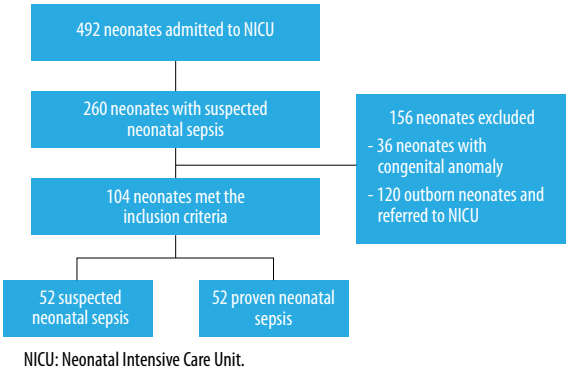
Ethical clearance in this study was approved by the Ethical Committee in Health Research of Dr. Soetomo General Academic Surabaya (ref. no. 1047/KEPK/III/2019).

Complete blood count performed by automated hematology analyzer and include white blood count (WBC) differential to evaluate the WBC based on light scattering characteristics. The ANC and absolute lymphocyte count were identified and counted in the WBC differential. The NLR is obtained by dividing the ANC by the absolute lymphocytes count recorded in the medical record manually. The diagnosis of neonatal sepsis is categorized into two groups based on blood culture results; proven neonatal sepsis if the blood culture results are positive and suspected neonatal sepsis if negative.

Data were analyzed using SPSS and presented as median and 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 the chi-square test. Total leukocyte count, ANC, absolute lymphocytes count, NLR, and CRP were analyzed using Mann-Whitney U test with significance defined as a p-value < 0.050. The NLR cut-off value was established using a receiver operating characteristic curve.

RESULTS

The number of inborn neonates admitted to the NICU during the six-month study period was 492, and 260 of them were suspected neonatal sepsis. A total 156 neonates were excluded from the study due to congenital abnormalities (n = 36) and were referred from other hospitals (outborn neonates, n = 120), so the total subjects who met the inclusion criteria were 104 neonates [Figure 1]. Neonatal sepsis in this study had an incidence of 10.6% with 52 (50.0%) neonates categorized as proven neonatal sepsis. Gram-negative bacteria dominated as the cause of neonatal sepsis (75.0%), with extended-spectrum β-lactamase producing *Klebsiella pneumoniae* (+) isolates making up 61.5%. Table 1 informs the subjects and laboratory characteristics of the subjects in this study. The median NLR (p = 0.018) and CRP (p = 0.001) in the proven neonatal



NICU: Neonatal Intensive Care Unit.

Figure 1: Flow diagram the research design.

Table 1: Subjects characteristics.

Variables	Proven neonatal sepsis n (%)	Suspected neonatal sepsis n (%)	Total n (%)	*p*-value
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 (25.0)	
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 g)	27 (51.9)	16 (30.8)	43 (41.3)	
Very low birth weight (< 1500 g)	20 (38.5)	17 (32.7)	37 (35.6)	
Extremely low birth weight (< 1000 g)	2 (3.8)	12 (23.1)	14 (13.5)	
Lubschenco score				0.512*
Appropriate for gestational age	39 (75.0)	36 (69.2)	75 (72.1)	
Small for gestational age	13 (25.0)	16 (30.8)	29 (27.9)	
Mode of delivery				0.222*
Spontaneous	16 (30.8)	22 (42.3)	38 (36.5)	
Sectio caesaria	35 (67.3)	28 (53.8)	63 (60.6)	
Others	1 (1.9)	2 (3.8)	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	13 (25.0)	28 (53.8)	41 (39.4)	
Late-onset neonatal sepsis	39 (75.0)	24 (46.2)	63 (60.6)	
Total leucocyte count, /mm^3, median (IQR)	11 700 (6405–17 590)	10 215 (4920–16 010)	11 265 (5745–17 265)	0.465**
Absolute neutrophil count, /mm^3, median (IQR)	7310 (4920–11 580)	5390 (2995–10 495)	6375 (3260–11 205)	0.155**
Absolute lymphocyte count, /mm^3, median (IQR)	1840 (1120–3145)	2155 (1270–3540)	2010 (1160–3245)	0.359**

**Chi-Square's test. **Mann-Whitney U test.*
IQR: interquartile range.

sepsis neonates was significantly higher than in the suspected group [Figure 2].

Neonates with NLR of 2.12 have an area under the curve (AUC) of 0.630 (95% confidence interval (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 cut-off 2.70 mg/dL, which was 0.690 (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 has high sensitivity of 80.8% but low specificity of 42.3%. The positive predictive value (PPV) and negative predictive value (NPV) were 58.3% and 68.8% with 62.5% accuracy. The combination of NLR and CRP have 75.0% sensitivity, 67.3% specificity, 69.6% PPV, 72.9%

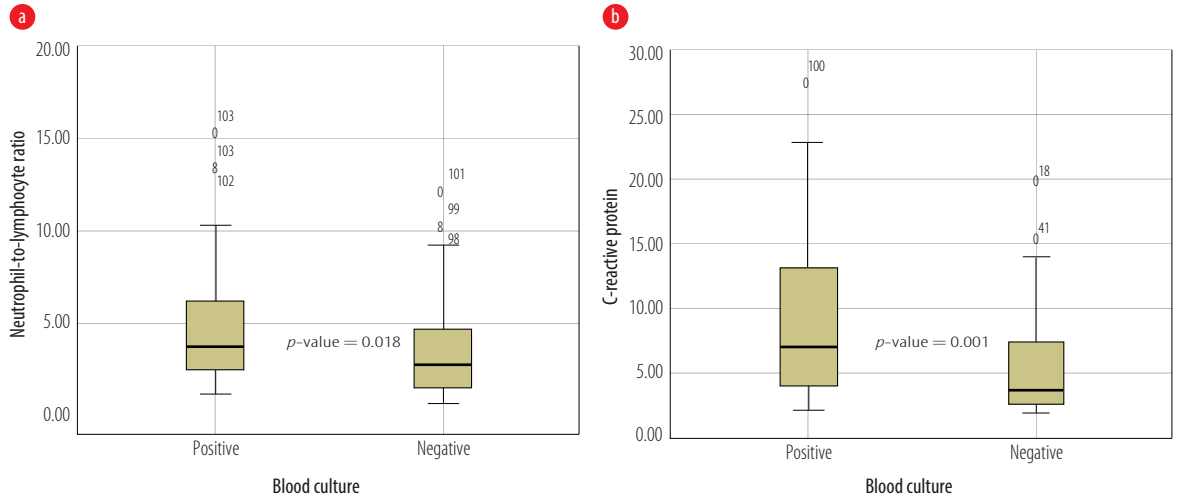


Figure 2: Box plot of **(a)** neutrophil-lymphocyte ratio and **(b)** C-reactive protein based on blood culture (Mann-Whitney U test).

NPV and 71.2% 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 CRP > 2.70 mg/dL, the risk of giving positive blood culture results is more than doubled (RR = 2.106, 95% CI: 1.396–3.179; p < 0.001).

DISCUSSION

Preterm and low birth weight (LBW) infants were the highest subjects to have neonatal sepsis in this study. This results were similar to the previous study reported in Denpasar in 2008 (50% preterm and 53.6% LBW neonates) and Surabaya in 2010 (77.78% preterm and 32% LBW neonates).[8,12] The incidence of neonatal sepsis is inversely related to birth weight and gestational age. It is mostly found in infants with very LBW < 1500 g and gestational age < 28 weeks.[5,13] Prematurity with sepsis as a complication was the leading cause of neonatal mortality. Higher neonatal death was also found at lower gestational age, with the majority born at < 26 weeks. The majority of neonatal death was noted in infants with a birth weight < 1000 g.[14] Our study included more males (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 75.0% of positive blood cultures were obtained in late-onset neonatal sepsis (LOS) and dominated by gram-negative bacteria. It is significantly 3.5-times more common than early-onset neonatal sepsis (EOS). LOS with positive

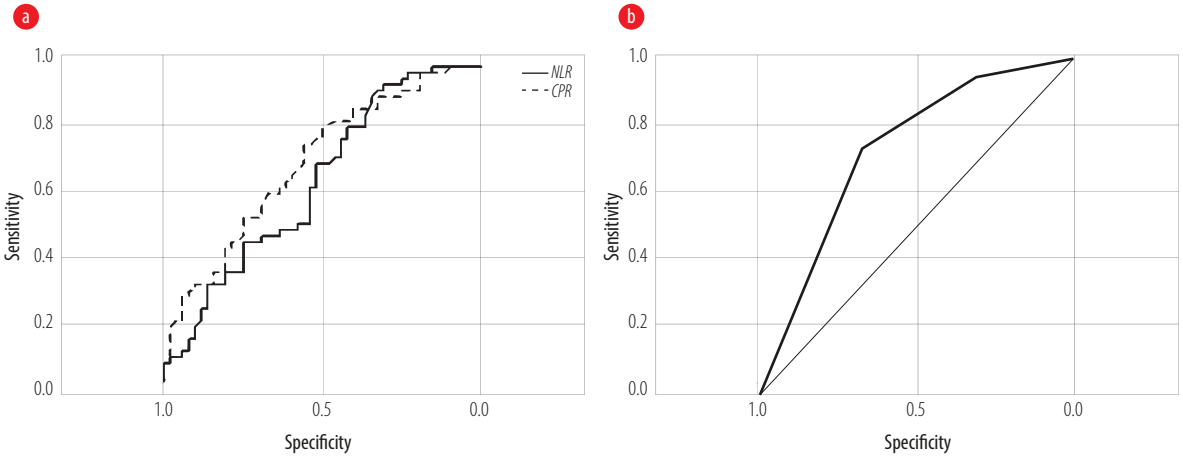


Figure 3: Area under curve **(a)** neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP) and **(b)** NLR combined with CRP as initial marker of neonatal sepsis.

blood cultures was also reported in previous studies by Shehab El-Din et al,[16] at 65% and Yusef et al,[20] at 72%. 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]The domination of gram-negative bacteria was also reported in a previous study, and it was found that intrapartum antibiotics, TPN duration, CVC duration, and mechanical ventilation duration were potential maternal and neonatal risk factors for late-onset gram-negative sepsis.[19] We did not evaluate the use of CVC, endotracheal tubes, and TPN in this study. Low humoral immune system in preterm neonates occurs because the transfer of transplacental immunoglobulin G from mother to fetus begins in the second trimester and peaks in the third trimester of pregnancy.[21,22]

Nearly 90% of neonatal sepsis with positive blood cultures in this study had normal leukocyte counts (4000–34 000/mm^3). Abnormal total leukocyte counts were only found in eight neonates (four EOS and four LOS) with total leukocyte counts < 4000/mm^3 and no neonates with total leukocyte counts > 34 000/mm^3. Sucilathangam et al,[23] also reported that normal total leukocyte counts were obtained in 12/14 (85.72%) neonates with positive blood culture results. A normal total leukocyte count cannot rule out the presence of neonatal sepsis because 50.0% of neonatal sepsis with positive blood cultures have a normal total leukocyte count.[9]

Higher median NLR was found in proven neonatal sepsis or neonates with positive blood cultures, and higher mean NLR in positive blood cultures (3.69±3.0) compared to negative blood cultures (1.56±1.83, $p < 0.001$).[1] Significantly, higher NLR compared to healthy neonates were also reported in several studies. The term neonatal sepsis group had a significantly higher NLR than healthy term neonates reported by Can et al,[24] with 2.88±0.16 and 0.21±0.12, respectively ($p = 0.02$), and Omran et al,[25] with 2.9±1.7 and 1.6±0.4, respectively ($p < 0.001$). Overall studies in neonatal sepsis groups have higher NLR values compared to NLR values previously reported by Hamiel et al,[11] in healthy neonates or the 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 response to fight bacterial infections and stimulates the process of emergency granulopoiesis. An increase in neutrophil counts is accompanied by a decrease in lymphopoiesis and monocytopoiesis and also a decrease in neutrophil apoptosis but an increase in lymphocyte apoptosis resulting in neutrophilia and lymphocytopenia.[1,26–28]

The NLR cut-off in this study was higher than in previous studies. The NLR 1.81 cut-off 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.[29] The NLR cut-off in this study was lower than in some previous studies but with higher sensitivity. Omran et al,[25] 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 et al,[30] obtained an NLR 9.4 cut-off 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 the study population.

The AUC of NLR and CRP are not statistically significantly different, but the best AUC is obtained if both are combined. Only one previous study also reported the best AUC of 0.79 (95% CI: 0.70–0.88) was found when NLR and CRP were combined compared to NLR or CRP alone. The study was conducted in term neonates aged 7–28 days old until 90 days old who experience serious bacterial infection.[11]

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, complications, and neonatal mortality due to infections, especially bacterial infections. This study's limitation is that it was collected at only one research center and we excluded neonates transferred from other hospitals. This study also did not include healthy neonates as a control group so the normal range value of NLR could not be known.

CONCLUSION

The NLR, by dividing the absolute count of neutrophils from lymphocytes from complete blood count, can be used as an alternative marker of neonatal sepsis, especially in developing countries. It is an easy and relatively inexpensive examination. Better detection of proven neonatal sepsis can be achieved using NLR when combined with CRP.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

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REFERENCES

1. Alkan Ozdemir S, Arun Ozer E, Ilhan O, Sutcuoglu S. Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? J Clin Lab Anal 2018 May;32(4):e22338.
2. Hasibuan BS. Comparison of microbial pattern in early and late onset neonatal sepsis in referral center Haji Adam Malik hospital Medan Indonesia. IOP Conf Ser Earth Enviromental Sci 2018;125(1):012053.
3. Edwards MS, Baker CJ. Sepsis in the newborn. In: Gershon A, Hotez P, Katz S, editors. Krugman's Infectious Disease of Children. 11th ed. Philadelphia, 2003. p. 545–563.
4. Polin RA; Committee on Fetus and Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics 2012 May;129(5):1006-1015.
5. Bany-Mohammed F. Sepsis. In: Gomella TL, Cunningham MD, Eyal FG, editors. Neonatology : management, procedures, on-call problems, disesase and drugs. 7th ed. New York, 2013. p. 865-874.
6. Coetzee M, Mbowane N, De Witt T. Neonatal sepsis: Highlighting the principles of diagnosis and management. S Afr J Child Heal 2017 Jul;11(2):99-103.
7. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med 2005 May;6(3) (Suppl):S45-S49.
8. Kardana IM. Incidence and factors associated with mortality of neonatal sepsis. Paediatr Indones 2011 May;51(3):144-148.
9. Gomella TL. Postdelivery antibiotic. In: Gomella TL, Cunningham MD, Eyal FG, editors. Neonatology: management, procedures, on-call problems, disesase and drugs. 7th ed. New York, 2013. p. 492-501.
10. Holub M, Beran O, Kaspříková N, Chalupa P. Neutrophil to lymphocyte count ratio as a biomarker of bacterial infections. Cent Eur J Med 2012 Jan;7(2):258-261.
11. Hamiel U, Bahat H, Kozer E, Hamiel Y, Ziv-Baran T, Goldman M. Diagnostic markers of acute infections in infants aged 1 week to 3 months: a retrospective cohort study. BMJ Open 2018 Jan;8(1):e018092.
12. Utomo MT. Neonatal sepsis in low birth weight infants in Dr. Soetomo General Hospital. Indones J Trop Infect Dis 2010 May-Aug;1(2):86-89.
13. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. Arch Dis Child Fetal Neonatal Ed 2015 May;100(3):F257-F263.
14. Abdellatif M, Ahmed M, Bataclan MF, Khan AA, Al Battashi A, Al Maniri A. The patterns and causes of neonatal mortality at a tertiary hospital in oman. Oman Med J 2013 Nov;28(6):422-426.
15. Ozkan H, Cetinkaya M, Koksal N, Celebi S, Hacımustafaoglu M. Culture-proven neonatal sepsis in preterm infants in a neonatal intensive care unit over a 7 year period: coagulase-negative Staphylococcus as the predominant pathogen. Pediatr Int 2014 Feb;56(1):60-66.
16. Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of neonatal sepsis and implicated pathogens: a study from Egypt. Biomed Res Int 2015;2015(1):509484.
17. Mahallei M, Rezaee MA, Mehramuz B, Beheshtirooy S, Abdinia B. Clinical symptoms, laboratory, and microbial patterns of suspected neonatal sepsis cases in a children's referral hospital in northwestern Iran. Medicine (Baltimore) 2018 Jun;97(25):e10630.
18. Murthy S, Godinho MA, Guddattu V, Lewis LE, Nair NS. Risk factors of neonatal sepsis in India: A systematic review and meta-analysis. PLoS One 2019 Apr;14(4):e0215683.
19. Abdellatif M, Al-Khabori M, Rahman AU, Khan AA, Al-Farsi A, Ali K. Outcome of late-onset neonatal sepsis at a tertiary hospital in Oman. Oman Med J 2019 Jul;34(4):302-307.
20. Yusef D, Shalakhti T, Awad S, Algharaibeh H, Khasawneh W. Clinical characteristics and epidemiology of sepsis in the neonatal intensive care unit in the era of multi-drug resistant organisms: A retrospective review. Pediatr Neonatol 2018 Feb;59(1):35-41.
21. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004 Jul;17(3):638-680.
22. Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr 2015 Feb;61(1):1-13.
23. Sucilathangam G, Amuthavalli K, Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palaniappan N. Early diagnostic markers for neonatal sepsis: comparing procalcitonin (pct) and c-reactive protein (crp). J Clin Diagn Res 2012 May;6(4):627-631.
24. Can E, Hamilcikan Ş, Can C. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. J Pediatr Hematol Oncol 2018 May;40(4):e229-e232.
25. Omran A, Maaroof A, Saleh MH, Abdelwahab A. Salivary C-reactive protein, mean platelet volume and neutrophil lymphocyte ratio as diagnostic markers for neonatal sepsis. J Pediatr (Rio J) 2018 Jan - Feb;94(1):82-87.
26. Wesche DE, Lomas-Neira JL, Perl M, Chung CS, Ayala A. Leukocyte apoptosis and its significance in sepsis and shock. J Leukoc Biol 2005 Aug;78(2):325-337.
27. Manz MG, Boettcher S. Emergency granulopoiesis. Nat Rev Immunol 2014 May;14(5):302-314.
28. Hwang SY, Shin TG, Jo IJ, Jeon K, Suh GY, Lee TR, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. Am J Emerg Med 2017 Feb;35(2):234-239.
29. Nugroho ID. Nilai diagnostik neutrophil to lymphocyte count ratio sebagai penanda awal pada neonatus terinfeksi [Thesis]. Universitas Sebelas Maret; 2018. Available from: https://eprints.uns.ac.id/40470/.
30. Ruslie RH, Tjipta DG, Samosir CT, Hasibuan BS. Bacterial pattern and role of laboratory parameters as marker for neonatal sepsis. IOP Conf Ser Earth Environ Sci 2018;125(1):012057.