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Manding MJM, Yala, ET. The Use of Absolute Neutrophil Count and Neutrophil-Lymphocyte Ratio as Predictors of Early Onset Neonatal Sepsis.
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ORIGINAL ARTICLE

THE USE OF ABSOLUTE NEUTROPHIL COUNT AND NEUTROPHIL-LYMPHOCYTE RATIO AS PREDICTORS OF EARLY ONSET NEONATAL SEPSIS

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ABSTRACT

Background: Neonatal sepsis contributes to significant morbidity and mortality. Blood culture, the gold standard in its diagnosis, has low sensitivity and is affected by multiple factors. Hence the need for markers derived from routine tests to improve diagnosis deserves further studies.

Objectives: This study aims to determine the association and optimal cut-off value and diagnostic performance of absolute neutrophil count (ANC) and neutrophil lymphocyte ratio (NLR) with early-onset neonatal sepsis in term neonates.

Methodology: This was a retrospective, analytical, single-center study of admitted patients from January 2016 to December 2021. Clinical factors were analyzed and NLR and ANC were derived from CBC and interpreted using the Manroe chart.

Results: Included were 200 neonates with a median birth AOG of 38 weeks. Microorganisms were isolated from nine of 154 neonates with blood culture, corresponding prevalence of 5.84% (95% CI 2.71–10.80). Initial CBC showed elevated mean WBC and 76.5% of neonates were considered to have elevated ANC. Optimal cut-off point of NLR for detecting culture-proven sepsis was 2.86, with a sensitivity of 88.89% (95% CI, 51.75–99.72%) and specificity of 36.55% (95% CI, 28.72–44.95%). The ANC gave the best balance of sensitivity and specificity with an accuracy of 75.50%.

Conclusions: The NLR demonstrated good discriminative ability for predicting clinical neonatal sepsis based on ANC. However, individually or simultaneously, these markers demonstrated poor discriminative ability for culture-proven neonatal sepsis in term neonates. ANC and NLR can be used to aid in the diagnosis of clinical neonatal sepsis.

KEYWORDS: *Absolute Neutrophil Count; Neutrophil to Lymphocyte Ratio, Neonatal Sepsis*

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INTRODUCTION

Neonatal sepsis (NS) is a significant global problem that contributes to significant morbidity and mortality in newborns, especially in developing countries.[1] As many as 1.6 million neonates die each year due to infection, with 60% deaths occurring in developing countries.[2] It is a clinical syndrome resulting from the invasion of microorganisms into the bloodstream that occurs in the first month of life.[3] Currently, the criteria for diagnosis of NS are mainly based on clinical signs,[4] however, these clinical symptoms resemble other noninfectious neonatal conditions, thus making the diagnosis of NS challenging.[5] The gold standard for diagnosis of NS is blood culture;[6] however, it takes 48 hours to obtain results and has low sensitivity. Studies show that positive cultures are only found in 30-40% of cases[7] and can be affected by several factors such as maternal antimicrobial treatment, inadequate volume of blood, and contamination.[8]

Other parameters used for the diagnosis of NS include complete blood count, C-reactive protein (CRP), and procalcitonin. However, interpretations are challenging because they vary significantly with the day of life and gestational age or has low sensitivity (CRP).[9] Thus, it is of critical importance to identify new biomarkers that will enable fast and reliable hematological scoring systems for sepsis in its early stages. The use of absolute neutrophil count (ANC) has improved the sensitivity in screening for neonatal bacterial disease.[10] A publication by Manroe *et al*., established a method for determining whether a neonate's neutrophil count should be considered neutrophilic, normal, or neutropenic.[11] The physiological immune response of circulating leukocytes to stressful events is characterized by a raised neutrophil count and decreased lymphocyte count. A microbial infection causes an increase of the total leukocyte and neutrophil counts and results in an inflammatory reaction. For this reason, these counts might be used as diagnostic markers of microbial infection.[12,13] Sepsis could give rise to elevated neutrophil counts and decreased lymphocyte counts resulting from infection of pathogenic microorganisms, indicating that septic neonates might have a higher neutrophil-to-lymphocyte ratio (NLR).[14] NLR is considered comparatively to be more stable than absolute neutrophil or lymphocyte counts as both neutrophil and lymphocyte counts are included in the calculation.[15] In a meta-analysis done by Huang, the prognostic value of NLR in sepsis concluded that it may be a helpful prognostic biomarker of patients with sepsis and that higher NLR values may indicate unfavorable prognoses in these patients.[16] However, there have been no studies that compare the utility of NLR in relation to ANC. The predictive value of ANC in early onset neonatal sepsis (EONS) combined with NLR, which is a more accessible, simpler and cheaper inflammatory marker, can lead to early treatment and reduce morbidity and mortality.

The objective of this study was to determine the association of ANC and NLR with EONS in term neonates, adjusting for AOG, birthweight and its appropriateness, and mode of delivery. Furthermore, to describe the demographic, clinical, and laboratory profile of septic neonates and to determine the optimal cut-off value and diagnostic performance (sensitivity, specificity, PPV, NPV, LR+, LR-, diagnostic accuracy, AUC); to predict EONS of the following: ANC, NLR, and simultaneous testing of ANC and NLR; and lastly to enumerate the isolates in blood cultures in early-onset sepsis of term neonates.

MATERIALS AND METHODS

Study Design and Population

This was a retrospective, observational, analytical, single-center study, and the data were obtained from chart review of neonates admitted to the Neonatal Intensive Care Unit of a tertiary training hospital in Tarlac City, Philippines, from January 2016-December 2021. Included in the study were term neonates born by spontaneous singleton delivery and cesarean section with a gestational age of 37 to 42 weeks via Ballard's scoring, appropriate for gestational age (AGA) diagnosed with EONS. Exclusion criteria include patients with incomplete data and those born under 37 weeks and more than

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42 weeks. Additionally, neonates with mothers who had preeclampsia and gestational diabetes mellitus; are small or large for gestational age, with significant congenital abnormalities, congenital heart diseases, hematologic diseases, and healthcare associated infections were likewise excluded due to their potential as confounders. Patients who were transferred from or to other institutions were also excluded.

Sample Size Computation

A minimum total of 481 term neonates with both CBC and blood cultures done to test for EONS is required for this study; this covers the sample size requirement for both NLR (n = 472) and ANC (n = 481), [17-19] based on a level of significance of 5%, precision at ±0.05, and an area under the curve of 0.586 of ANC to predict sepsis. A total of 472 term neonates with both CBC and blood cultures done, are required for this study based on a level of significance of 5%, precision at ±0.05, and an area under the curve of 0.63 of NLR to predict sepsis. The AUC-based sample size formula was based on Hajian-Tilaki *et al.,* 2014.

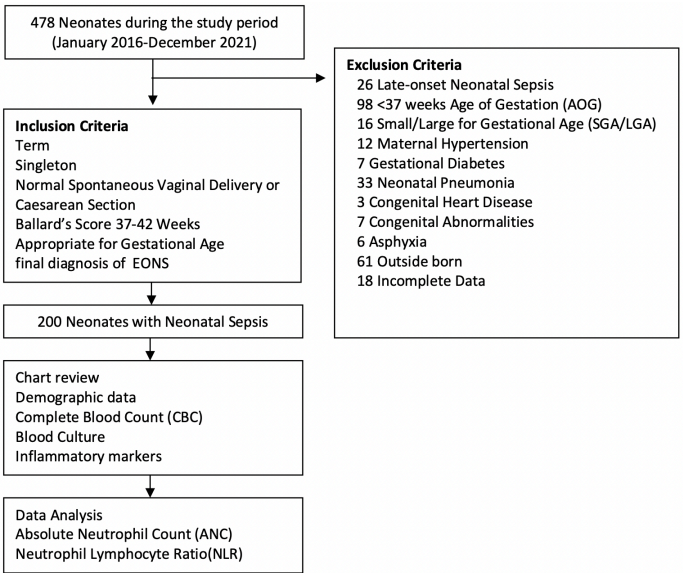

Figure 1. Study Enrollment – of the 478 neonates admitted in the study period, 200 met the study inclusion criteria. Analysis was performed to determine the relationship of EONS with NLR and ANC.

Ethical Consideration

The study protocol was approved by the University of the East Ramon Magsaysay Memorial Medical Center, Inc. Research Institute for Health Sciences Ethics Review Committee. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. This study was partially funded by the Pediatric Infectious Disease Society of the Philippines. The authors declare no conflict of interest.

Data Sampling and Collection

Digital medical records of patients who fulfilled the criteria for the study were retrieved and reviewed. Data collected were entered with a researcher-developed data collection form that included the demographic profile, prenatal and neonatal history, clinical signs, complete blood count, and other relevant laboratory results. Patient data were de-identified into an identification code. All existing data were stored electronically in a password-protected computer for five years, where access to data will only be accessible to the study proponents**.**

Statistical Analysis

Descriptive statistics were used for categorical variables- frequency and percentage. Shapiro-Wilk or test was used to determine the normality distribution. Continuous quantitative data that meet normality assumption were summarized using mean and standard deviation (SD), while those that do not were described using median and range. Continuous variables that are normally distributed were compared using an independent t-test. Otherwise, the non-parametric Mann-Whitney U test was used. For categorical variables, Chi-square test was used to compare the proportion between two groups. If the expected percentages in the cells are less than 5%, Fisher's Exact Test was used.

Receiver operating characteristic (ROC) analysis was performed, and Youden's J index was defined for all points along the ROC curve. The maximum index value (best balance of sensitivity and

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specificity) was used to select the optimal cut-off point. ROC curves were plotted, giving areas under the curve (AUROC), which could range from 0.5 (no discriminative ability) to 1.0 (perfect discriminative ability). The optimal cut-off value's sensitivity, specificity, predictive values, likelihood ratios, and diagnostic accuracy in detecting EONS were determined. Crude and adjusted odds ratios and the corresponding 95% confidence intervals from logistic regression were computed to determine the associations of ANC and NLR (based on Manroe chart and optimal cut-off values from AUROC) with EONS.

All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05α-level of significance. STATA 15.0 (StataCorp SE, College Station, TX, USA) was used for data analysis.

ANC is computed as *WBC x Neutrophil x 10*, while NLR is computed by dividing neutrophil count and lymphocyte count.

RESULTS

Our study population included 200 neonates with a median of 38 (range 37-41) weeks AOG. Subjects comprised 57% males, and 76% were delivered via cesarean section (Table 1). The birth length and weight medians were 49 (range 39-59) cm and 2975 (range 2020-3860) grams, respectively. The initial CBC was obtained at a median of the 8th hour of life. The most common clinical signs compatible with sepsis were respiratory distress (32%), poor feeding, (23%), vomiting (22%) and jaundice (11%).

Pathogenic organisms were isolated from 9 of 154 neonates with a blood culture. The corresponding prevalence was 5.84 (95% CI 2.71–10.80) per 100. Microbial growths belonged to the genera *Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Pantoea*, *Shigella*, *Staphylococcus*, and *Streptococcus* from initial blood culture results.

Table 1. Neonatal characteristics of all septic neonates admitted in a Neonatal Intensive Care Unit from January 2016-December 2020

	All (n=200)	Culture-positive (n=9)
	Median (Range);	Frequency (%)
AOG, weeks	38 (37-41)	39 (37-39)
Sex		
Male	114 (57.00)	4 (44.44)
Female	86 (43.00)	5 (55.55)
Mode of delivery		
Cesarean section	152 (76.00)	8 (88.89)
Normal spontaneous	48 (24.00)	1 (11.11)
Birth length, cm	49 (39-59)	50 (45-57)
Birthweight, grams	2975 (2020-3860)	3000 (2300-3500)
2001-2500	30 (15.00)	2 (22.22)
2501-3000	88 (44.00)	3 (33.33)
3001-3500	69 (34.50)	4 (44.44)
3501-4000	13 (6.50)	0
Circumference, cm		
Head	34 (30-37)	34 (31-37)
Chest	32 (24-36)	32 (30-35)
Abdominal	30 (24-36)	29 (26-34)
CBC timing, hour of life	8 (1-144)	8 (6-48)
Clinical signs of sepsis		
Respiratory distress	64 (32.00)	4 (44.44)
Vomiting	45 (22.50)	2 (22.22)
Poor feeding	47 (23.50)	1 (11.11)
Jaundice	22 (11.00)	1 (11.11)
Cyanosis	21 (10.50)	1 (11.11)
Tachycardia	1 (0.50)	0
Apnea	0	0
Bradycardia	0	0
Lethargy	0	0
Isolated pathogen		
Pseudomonas stutzeri	-	2 (22.22)
Acinetobacter baumannii	-	1 (11.11)
Burkholderia cepacia	-	1 (11.11)
CoNS	-	1 (11.11)
Pantoea agglomerans	-	1 (11.11)
Shigella spp.	-	1 (11.11)
Staphylococcus epidermidis	-	1 (11.11)
Streptococcus agalactiae	-	1 (11.11)

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Initial CBC showed elevated mean (±SD) WBC count of 26.25 ± 8.37 x10^9/l and ANC of 17647.88 ± 6593.41 x10^6/l (Table 2). 153 (76.5%) neonates were considered to have elevated ANC using the Manroe chart. The average neutrophil and lymphocyte differential counts were 65.61% and 21.72%, respectively, and the median NLR was 3.35 (range 0.51-11.11).

Table 2. Initial CBC and other parameters, Procalcitonin and C-Reactive Protein of all septic neonates admitted in a Neonatal Intensive Care Unit from January 2016- December 2020

Parameters	All (n=200)	Culture-positive (n=9)
	Mean ± SD; Median (Range); Frequency (%)	
Procalcitonin, ng/mL [n=6]		
<0.5	0	-
≥0.5	6 (100)	-
CRP, mg/L [n=3]		
<1.0	1 (33.33)	-
1.0-3.0	1 (33.33)	-
>3.0	1 (33.33)	-
Complete Blood Count		
Hemoglobin g/dL	17.58 ± 2.70	17.78 ± 2.99
Hematocrit %	51.92 ± 8.48	52.41 ± 8.70
Platelet x1000/µL	280.56 ± 72.95	283.44 ± 60.35
WBC x1000/µL	26.25 ± 8.37	29.89 ± 8.36
Neutrophils, %	65.61 ± 9.84	68.04 ± 6.76
Lymphocytes, %	21.72 ± 9.14	20.57 ± 7.83
NLR	3.35 (0.51-11.11)	3.68 (1.30-6.35)
ANC cells/µL	17647.88 ± 6593.41	20433.56 ± 6297.76
ANC classification		
Normal	47 (23.50)	1 (11.11)
Elevated	153 (76.50)	8 (88.89)

ANC and NLR, individually or simultaneously, demonstrated poor discriminative ability for culture-proven NS in term neonates (n=9/154 neonates), with AUCs ranging from 0.569 to 0.636 (Table 3, Figure 1).

Table 3. Predictive abilities of CBC-derived values for culture-proven EOS in term neonates (n=154)

	AUC (95% CI)
ANC	0.635 (0.430–0.840)
NLR	0.569 (0.399–0.739)
ANC and NLR	0.636 (0.422–0.850)

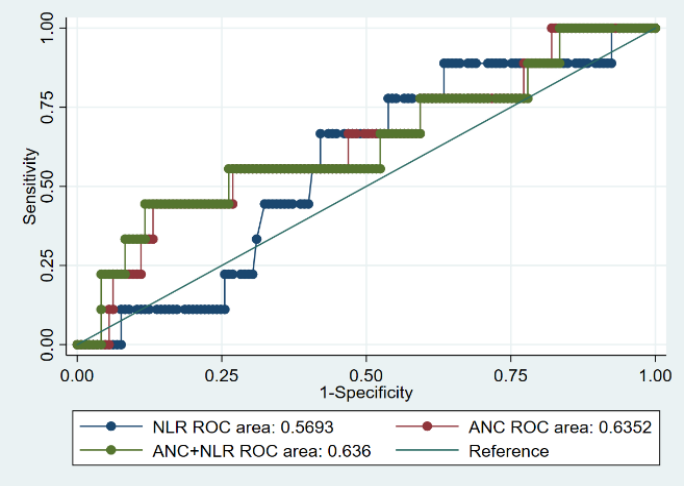

Figure 2. ROC curves of ANC and NLR, individually and simultaneously, to predict culture-proven EONS.

The optimal cut-off point of NLR for detecting culture-proven NS was 2.86, and values greater than or equal to this gave a sensitivity of 88.89% (95% CI, 51.75–99.72%) and specificity of 36.55% (95% CI, 28.72–44.95%). Its overall accuracy was 39.61% (95% CI, 31.83–47.80%) (Table 4). For elevated ANC by Manroe chart, the sensitivity was similar at 88.89% (95% CI, 51.75–99.72%), specificity lower at 25.52% (95% CI, 18.65–33.42%), and overall accuracy lower at 29.22% (95% CI, 22.18–37.08%).

Table 4. Diagnostic performance of elevated ANC and NLR in screening for culture-proven EOS in term neonates (n=154)

	Elevated ANC	NLR ≥ 2.86
	Point Estimate (95% CI)	
Sn, %	88.89 (51.75–99.72)	88.89 (51.75–99.72)
Sp, %	25.52 (18.65–33.42)	36.55 (28.72–44.95)
PPV, %	6.90 (5.45–8.68)	8.00 (6.27–10.15)
NPV, %	97.37 (85.10–99.58)	98.15 (89.19–99.71)
Positive LR	1.19 (0.93–1.53)	1.40 (1.08–1.82)
Negative LR	0.44 (0.07–2.82)	0.30 (0.05–1.95)
Accuracy, %	29.22 (22.18–37.08)	39.61 (31.83–47.80)

Sn – Sensitivity; Sp – Specificity; LR – Likelihood Ratio

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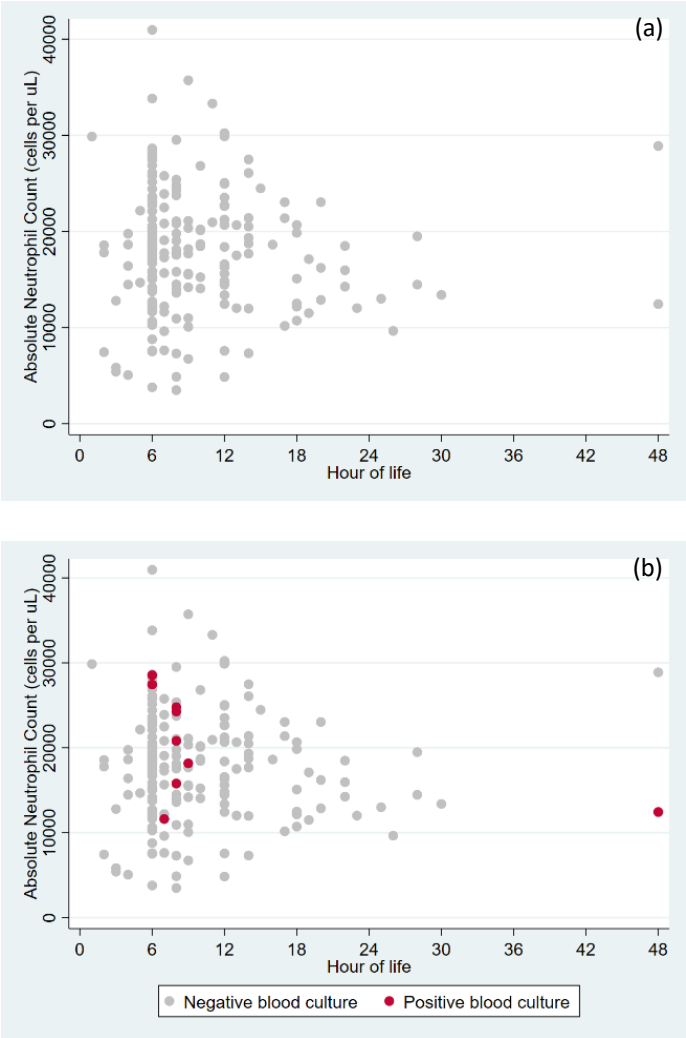

Figure 3. Absolute neutrophil counts were plotted according to hour of life when CBC was taken. (a) All neonates (n=200); (b) Neonates with blood culture (n=154). Red points indicate those that showed growth on culture.

Table 5. Association of ANC classification and NLR with culture-proven EOS in term neonates (n=154)

	Crude OR (95% CI)	*P*	Adjusted* OR (95% CI)	*P*
ANC classification				
Normal	Reference	-	Reference	-
Elevated	2.74 (0.33–22.65)	.349	1.30 (0.12–13.77)	.825
NLR				
<2.86	Reference	-	Reference	-
≥2.86	4.61 (0.56–37.87)	.155	4.09 (0.40–42.22)	.237

*Controlled for birth AOG, sex, and mode of delivery

NLR demonstrated good discriminative ability for predicting clinical NS using the ANC classification based on the Manroe chart in term neonates (AUC = 0.8580; 95% CI 0.8005 to 0.9156) (Figure 4).

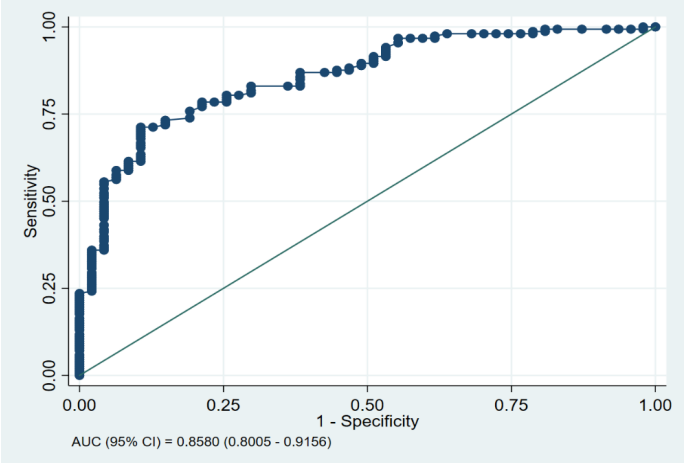

Figure 4. NLR discriminative ability for predicting clinical neonatal sepsis using the ANC classification

Youden's J index was used to determine the optimal cut-off point for NLR in predicting clinical NS using the ANC classification based on the Manroe chart, which gives the point with the best balance of sensitivity and specificity. NLR ≥ 3.06 had a fair sensitivity of 71.24%, high specificity of 89.36%, and an accuracy of 75.50% (Table 6).

Table 6. Diagnostic performance of optimal cut-off point of NLR in screening for clinical sepsis based on ANC classification from the Manroe chart (n=200)

	NLR
Disease prevalence, % (95% CI)	76.50 (70.00–82.19)
Sensitivity, % (95% CI)	71.24 (63.38–78.26)
Specificity, % (95% CI)	89.36 (76.90–96.45)
PPV, % (95% CI)	95.61 (90.44–98.05)
NPV, % (95% CI)	48.84 (42.20–55.52)
Positive likelihood ratio (95% CI)	6.70 (2.91–15.43)
Negative likelihood ratio (95% CI)	0.32 (0.25–0.42)
Accuracy, % (95% CI)	75.50 (68.94–81.29)

Term neonates with NLR ≥ 3.06 had about 20 to 24-fold increase in odds to have clinical NS based on the ANC classification from the Manroe chart, whether by crude association or after adjusting for AOG, sex, and mode of delivery.

Table 7. Association of NLR with clinical NS based on ANC classification from the Manroe chart (n=200)

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	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
NLR				
<3.06	Reference	-	Reference	-
≥3.06	**20.81 (7.72–56.06)**	**<.001**	**24.20 (8.61–68.02)**	**<.001**

Adjusted analysis accounted for the following variables: AOG, sex, mode of delivery

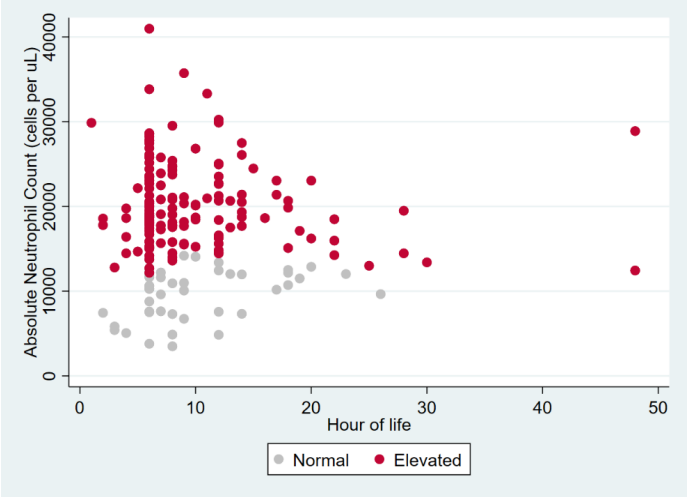

Figure 5. ANC classification based on Manroe chart

DISCUSSION

Our study showed that the most common clinical signs observed in patients with neonatal sepsis in this study included respiratory distress (32%), poor feeding (23%), and jaundice (11%). Microorganisms were isolated from nine of 154 neonates with blood culture, a corresponding prevalence of 5.84% (95% CI 2.71–10.80). Initial CBC showed elevated mean WBC and 76.5% of neonates considered to have elevated ANC. The optimal cut-off point of NLR for detecting culture-proven sepsis was 2.86, with a sensitivity of 88.89% (95% CI, 51.75–99.72%) and specificity of 36.55% (95% CI, 28.72–44.95%). The ANC gave the best balance of sensitivity and specificity with an accuracy of 75.50%.

The clinical profile findings are in accordance with the study conducted by Nasser et al., where respiratory distress was the most frequent clinical sign of EONS at 60%, followed by tachycardia (10%), jaundice, lethargy, and apnea (6.7 %), and lastly poor feeding, bradycardia, and cyanosis by 3.3% .[20] On the other hand, Can *et al,.* found the main presenting signs were apnea and bradycardia (31%), followed by tachycardia (29%), jaundice, poor feeding, and lastly, respiratory distress (5%).[21]

Albeit the gold standard for the diagnosis of sepsis, as shown by the low prevalence of culture-proven sepsis in our study, the rate of yield of blood culture is low [22] especially in developing countries wherein clinically diagnosed sepsis is present in 49-170 per 1000 births and the culture proven sepsis was at 16 per 1000 live births.[23] Results takes up to 48 hours to obtain which may be affected by multitude of factors such as inadequate blood volume, contamination and maternal antimicrobial use.[22] In our study, we yielded no predominant microbial growth but instead included *Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Pantoea*, *Staphylococcus*, and *Streptococcus* in comparison to commonly reported microorganisms in EONS which includes *S. agalactiae* and *E.coli*.[20] The blood culture were obtained from the first day of life and the results may have been influenced multiple factors.

CBC is a conventional laboratory test to aid in the diagnosis of sepsis and we derive multiple inflammatory markers which includes total leukocyte count, immature to total neutrophil count and ANC. However, WBC has been of little clinical use due to its broad variation hence Manroe chart was widely utilized[24] which shows the total neutrophil count in the first 60 hours of life.[10] In our study, initial CBC showed an elevated (±SD) WBC count of 26.25 ± 8.37 $x10^9$/l and ANC of 17647.88 ± 6593.41 $x10^6$/l. 153 (76.5%) neonates were considered to have elevated ANC as plotted in the chart.

NLR is considered more stable than absolute neutrophil or lymphocyte counts since both neutrophils and lymphocytes are included in the computation.[22] Normal NLR values in healthy neonatal or pediatric populations have been reported with an average value of 0.52- 0.91.[25] In our study, the cut-off point for NLR in predicting clinical NS using ANC classification based on the Manroe chart was ≥3.06, which had fair sensitivity at 71.24% and high specificity at 89.63%. In contrast, the optimal cut-off point of NLR for detecting culture-

proven NS was lower at 2.86, and values greater than or equal to this gave a sensitivity of 88.89% and specificity of 36.55%. Various studies reported cut-off points close to our determined value: Omran *et al.,* identified that NLR, at a cut-off value of 2.7, had a sensitivity of 80% and specificity of 57.1%[26], while Wilar *et al.,* found that the mean NLR from EONS and non-EONS group was 2.82±2.29 (sensitivity of 83.3% and specificity 57.1%) and 0.82±0.32, respectively.[27] In a similar study population, higher mean NLR was found in those with positive blood cultures at 3.69 (±3.0) than those with negative blood cultures at 1.56 (±1.83).[28] These studies had similar findings which showed a higher NLR value on septic neonates versus the control group. On the other hand, Can *et al.,* found a higher predictive cut-off value of NLR for EONS at 6.76 (sensitivity 97.4%; specificity 100%) compared in our study,[21] while another study determined a lower predictive NLR cut off value in neonates with NS at 1.81 with 86.1% sensitivity, 85.1% specificity, 68.9% PPV, and 94.1% NPV.[29]

Several studies reported significantly higher NLR in septic neonates compared to healthy neonates. Comparing the determined cut-off point of NLR at 2.86 for detecting culture-proven NS in those with elevated ANC by Manroe chart, the sensitivity was similar at 88.89% specificity lower at 25.52%. Furthermore, NLR demonstrated good discriminative ability to predict clinical NS using ANC classification when compared using the classification based on the Manroe chart. In a study done by Uri *et al.,* results show that the markers ANC and NLR have similar sensitivity in identifying serious bacterial disease.[31] Term neonates with NLR >3.06 had about 20 to 24 fold increase in odds of clinical NS. However, NLR showed no significant association with culture-proven EONS outcomes. This is in contrast with the study done by Sumitro *et al.,* where neonates with an NLR ≥2.12 have almost twice the risk of giving a positive blood culture, and when combined with CRP≥2.70mg/dL, the risk of providing positive blood culture results is more than doubled.[32] On the other hand, one study showed that NLR when combined with CRP would have better accuracy in diagnosing sepsis.[28] Inclusion of NLR as a new diagnostic marker for EONS is reinforced in this study in context with previous studies and the management of sepsis are mainly based on clinical signs which are nonspecific.[4] Blood cultures lack sensitivity due to specific characteristics of the neonatal population[33] and results can be affected by multiple factors. As a result, a combination of findings may then explain the poor discriminative ability in culture proven sepsis, but a good discriminative ability in clinically diagnosed NS.

Interpretations of our findings should take into account study limitations. The study was done in a single center with relatively small sample size in a retrospective manner. Also, the study did not include healthy neonates in comparing the utility of NLR and ANC. Some subjects also did not have culture results or culture was not done which may have contributed to the low yield of positive results.

CONCLUSION AND RECOMMENDATIONS

We demonstrated the good discriminative ability of NLR in predicting clinical NS based on the ANC of Manroe. However, ANC and NLR, individually or simultaneously, demonstrated poor discriminative ability for culture-proven NS in term neonates. ANC and NLR, can be used as an additional marker of infection to aid in the diagnosis of clinical NS and can be further evaluated in a prospective analysis with larger population and in subsets of patients with culture proven sepsis. A comparison of its diagnostic performance may further be established when compared to healthy neonates and may also be correlated with other inflammatory markers such as ESR, CRP, and procalcitonin.

REFERENCES

1. Alkan Ozdemir S, Arun Ozer E, Ilhan O, et al. Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? J Clin Lab Anal. May 2018; 32:e22338.
2. Haque KN, et al. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. May 2005; 6: S45-S49.
3. Rusell AB, Isaacs D. Rennie JM, Robertson NR. Infection in the newborn. 5th edition, London: Churchill Livingston; 2012.p.1013-1031.
4. Mukhopadhyay S, Puopolo KM. Risk assessment in neonatal early onset sepsis. Seminars in Perinatology 2012 [Cited

2021 Sep 01];36. Available from https:// doi.org/: 10.1053/j.

5. Li T, Dong G, Zhang M, et al. Association of neutrophil-lymphocyte ratio and the presence of neonatal sepsis. J of Immunol Research 2020 [Cited 2021 Sep 01];Available from https://doi.org/10.1155/2020/765071.
6. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics. Pediatric Critical Care Medicine 2005 [Cited 2021 Sep 01]; 6. Available from https://doi.org/10.1097/01.PCC.0000149131.72248.E6.
7. Ferrieri P, Wallen LD, Gleason CA, et al. Avery's disease of the newborn. Philadelphia (PA) : Saunders Elsevier, 2012. p.538-550.
8. Iroh Tam PY, Bendel CM. Diagnostics for neonatal sepsis: current approaches and future directions. Pediatric Research 2017 [Cited 2021 Sep 15];82. Available from https://doi.org/10.1038/pr.2017.134.
9. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal Infectious Diseases: Evaluation of Neonatal Sepsis. North Am : Pediatr Clin, 2013, Vol. 367.
10. Manroe BL, Weinberg AG, Rosenfeld CR, et al. The neonatal blood count in health and disease.I. Reference values for neutrophilic cells. The Journal of Pediatrics 1979 [Cited 2021 Oct 03];95. Available from https://doi.org/10.1016/s0022-3476(79)80096-7.
11. Das A, Ray S, Chattopadhyay A, et al. Gestation-wise reference ranges of neutrophil counts in Indian newborns. Oman Medical Journal 2019 [Cited 2021 Mar 28]; 34. Available from https://doi.org/10.5001/omj.2019.25.
12. Elawady S, Botros SK, Sorour AE, et al. Neutrophil CD64 as a Diagnostic Marker of Sepsis in Neonates. Pediatr Infect Dis J 2014 https://doi.org/10.2310/JIM.0000000000000060
13.. Yoon NB, Son C, Um SJ. Role of the neutrophil-lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community-acquired pneumonia. Ann Lab Med 2013 Mar;33(2):105-110.
14. Shen XF, Cao K, Jiang JP, et al. Neutrophil dysregulation during sepsis: an overview and update. J of Cellular and Molecular Medicine 2017; 21: p1687-1697.
15. Dirican A, Kucukzeybek B, Alacacioglu A, et al. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? International Journal of Clinical Oncology. 2015 [Cited 2021 Mar 28]; 20. Available from https://doi.org/doi: 10.1007/s1014.
16. Huang Z, Fu Z, Huang W, et al. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med 2020 [Cited 2021 Mar 03]; 38. Available from https://doi.org/doi: 10.1016/j.ajem .
17. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. *J Biomed Inform* 2014; 48:193 – 204. Accessed from https://www.ncbi.nlm.nih.gov/pubmed/24582925.
18. Li T, Dong G, Zhang M, Xu Z, Hu Y, Xie B, Wang Y, Xu B. Association of Neutrophil–Lymphocyte Ratio and the Presence of Neonatal Sepsis. Journal of Immunology Research. 2020 Dec 2;2020.
19. Hornik CP, Benjamin DK, Becker KC, Benjamin Jr DK, Li J, Clark RH, Cohen-Wolkowiez M, Smith PB. Use of the complete blood cell count in early-onset neonatal sepsis. The Pediatric infectious disease journal. 2012 Aug;31(8):799
20. Nasser MM, Afia AA, El-Khatib GZ, Ibrahim MI. The value of neutrophil to lymphocyte ratio and procalcitonin level for detecting early-onset neonatal sepsis. Journal of Pediatrics 2020 [Cited 2021 Jan 02]; 23. Available from https://doi.org/10.21608/azjp.2020.85895.
21. Can E, Hamilcikan Ş, Can C. et al. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. J Pediatr Hematol Oncol 2018 [Cited Jan 27]; 40. Available from PMID: 29219889.
22. Li T, Dong G, Zhang M, et al. Association of neutrophil lymphocyte ratio and the presence of neonatal sepsis. J of Immunology Research 2020 [Cited 2021 Mar 28]. Available from https://doi.org/10.1155/2020/7650713.
23. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. The New England Journal of Medicine 2005; 352: 1-13.
24. Stearns-kurosawa, DJ, Osuchwoski M, Valentine C, et al. The pathogenesis of sepsis. Annual Review of Pathology 2011; 6:19-48.
25. Hamiel U, Bahat H, Kozer E, et al. Diagnostic markers of acute infections in infants aged 1 week to 3 months: A retrospective cohort study. BMJ Open 2018; 8:e018092.
26. 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 2018 [Cited 2021 Jan 27]; 94. Available from https://doi.org/10.1016/j.jped.2017.03.006. Epub 201.
27. M, Weitzman. Diagnostic utility of white blood cell counts and differential counts. Am J Dis Child 1975; 129:1183.
28. Sumitro KR, Utomo1 MT, Widodo AD. Neutrophil-to-lymphocyte ratio as an alternative marker of neonatal sepsis in developing countries. Oman Medical Journal 2021 [Cited 2021 Jan 27]; 36. Available from https://doi.org/10.5001/omj.2021.05.
29. Nugroho ID, Hidayah D, Yulidar H. Diagnostic value of neutrophil to lymphocyte ratio as an early marker in infected neonates. Universitas Sebelas Maret Surakarta / Regional General Hospital, April 2018. [Cited 2021 Nov 15]. Available from https://eprints.uns.ac.id/40470/

30. Harmansyah H, Alasiry E, Daud D. Absolute neutrophil count as predictor of early onset sepsis. Clinical Medicine Research 2015; 4. 87.10.11648/j.cmr.20150403.15.
31. Uri H., Hilla B., Eran K., Yotam H.,. Diagnostic markers of acute infections in infants aged 1 week to 3 months: a retrospective cohort study. 2018, [Cited 2021 Nov 15]. BMJ Open.
32 Huang Z, Fu Z, Huang W, et al. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: a meta-analysis. The American Journal of Emergency Medicine 2020; 38: [Cited 2021 Mar 15]
33. Zea-Vera A, Ochoa T J. Challenges in the diagnosis and management of neonatal sepsis. Journal of Tropical Pediatrics 2015 [Cited 2021 Feb 02]; 61. Available from https://doi.org/10.1093/tropej/fmu079.