MAK HILL Publications International Journal of Tropical Medicine

Case Study
doi: 10.59218/makijtm.2024.2.45.49

Evaluation of Neutrophil-Lymphocyte Ratio as a Marker for Sepsis in Neonates: A Case Control Study

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OPEN ACCESS

Key Words
Blood culture, neonatal sepsis, newborn, sepsis screen

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Received: 25 February 2024
Accepted: 10 April 2024
Published: 20 April 2024

Citation: Dasara Gururaju, R. Akhila, R. Ramu, 2024. Evaluation of Neutrophil-Lymphocyte Ratio as a Marker for Sepsis in Neonates: A Case Control Study Int. J. Trop. Med., 19: 45-49, doi: 10.59218/makijtm.2024.2.45.49

ABSTRACT

Sepsis is one of the most common causes of neonatal mortality and morbidity Diagnosis of sepsis in neonates is challenging due to overlapping of signs and symptoms. Currently blood culture and sepsis screen are used for diagnosis. Blood culture is the gold standard but its usefulness is limited due to low positivity, delay in reporting. Sepsis screen has variable sensitivity and specificity. To overcome these limitations, neutrophil- lymphocyte ratio can be used in diagnosis. To assess neutrophil-lymphocyte ratio as a marker of neonatal sepsis. To calculate sensitivity and specificity of NLR in diagnosing neonatal sepsis. This case-control study was conducted from June 202-2022 in NICU of tertiary care center, Karnataka. 198 Neonates with signs and symptoms of sepsis and/or risk factors of sepsis were included. Weight and gestational age matched 198 healthy neonates served as control. Investigations include Complete blood count, blood culture and sepsis screen were sent. NLR was compared between cases and controls. Sensitivity and specificity of NLR was calculated. Data of both the groups were compared using independent t-test. ROC was used to derive the cut off of NLR. Demographic details are homogenous in both cases and control group. Mean NLR in cases is 2.6+/-1.5 and in controls is 1.3+/-0.2. On comparing mean NLR between cases and controls statistically significant difference is noted with p<0.001. The area under curve (AUC) for NLR in diagnosing neonatal sepsis was 0.83. The NLR cut off of >1.5 had sensitivity of 78.8%, specificity of 75.8%, positive predictive value (PPV) of 76.5% and negative predictive value of 78.1% This study demonstrated strong correlation between NLR and neonatal sepsis. To conclude, NLR is cheaper and widely available markers in diagnosing neonatal sepsis with resource limited setting.

INTRODUCTION

Sepsis is a clinical syndrome characterized by signs and symptoms of infections and the isolation of pathogens in the blood in child less than 28 days of life[1]. It is one of the most common causes of neonatal mortality and morbidity. It includes septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection It can be early onset (<72hrs) or Late onset. It is more common in pretem and low birth babies[2]. 1.6 million neonatal deaths occur in each year of which 60% occur in developing countries[3].Hence early diagnosis and prompt treatment is necessary in reducing mortality and morbidity. Currently blood culture and sepsis screen are in use to diagnose sepsis in neonates. Even though Blood culture is the gold standard test to diagnose sepsis but delay in reporting and often yield negative results[4] Sepsis screen include total leucocyte count, absolute neutrophil count (ANC), immature/total neutrophil ratio(I/T), C-reactive protein and micro ESR has variable sensitivity and specificity ranging from 50-90%[5]. All these investigations require higher cost and may not available in all peripheral settings in developing countries like India. To overcome these limitations Neutrophil-lymphocyte ratio(NLR) can be used which is inexpensive and a part of routine complete blood counts by autoanalyser. Studies on NLR's usefulness in diagnosing neonatal sepsis in limited. Hence present study was conducted to analyze usefulness of NLR values as early marker in diagnosing sepsis in neonates.

MATERIALS AND METHODS

This was an observational case control study. Conducted between june 2022-2022 for a period of 6 months in NICU of department of pediatrics in a tertiary care center, Karnataka. The study was approved by institutional ethical committee [IEC no. SIMS/IEC/448]]. All neonates admitted to NICU during the study period with signs and symptoms of sepsis and/or neonates born to mothers with risk factors of sepsis like fever, foul smelling discharge, uterine tenderness, leaking per vagina >24hrs, were included in the study as cases. For each case, weight and gestational age matched healthy neonate with no signs and symptoms of sepsis were taken as control. Informed consent was taken from parents of cases and controls.

Sample Size: Sample size was calculated based on expected sensitivity of 85%, absolute precision of 5% at 95% significance level and 195 cases were required. Sample size was calculated using Open Epi software (version 3.1)[6]. Sign and symptoms of sepsis include body temperature of >38.5 degree Celsius or < 36 degree Celsius, reduced urine output(<0.5ml/kg/hr), hypotension(<5th centile for gestational age), cardiovascular instability-bradycardia or tachycardia, mottled skin, impaired peripheral circulation, petechial rashes, sclerema, apnoea, tachypnoea (>60 breaths/min), feeding intolerance, poor sucking, abdominal distension, irritability, lethargy and hypotonia[7]. Blood investigations including complete blood count, sepsis screen and blood culture, were sent for testing before starting antibiotics. Under aseptic precaution 1 ml of venous blood was taken for culture and observed at least after 72 hrs for any growth. Sepsis screen include total leucocyte count (TLC-<5000/cumm), Absolute neutrophil count (ANC<1800/cumm), C-reactive protein (CRP>10), MICRO ESR (>15mm in 1st hr) and Immature to total leucocyte ratio (I: T>0.2). At least 2 abnormal parameters were taken to define sepsis screen[8] NLR values were compared between cases and controls and p value was derived. Based on blood culture and sepsis screen results cases were divided into 3 groups i.e. Culture positive, screen positive and clinical sepsis. Culture positive sepsis is characterized by positive blood culture. Screen positive sepsis is characterized by blood culture negative, but presence of at least two laboratory signs of sepsis screen were positive .Clinical sepsis is defined as presence of at least two clinical symptoms, but neither laboratory signs nor positive blood culture positive[9]. NLR was calculated in each group. Based on onset of symptoms cases are divided into early onset and late onset sepsis. Early onset sepsis and late onset sepsis are characterised by onset of symptoms within 72 hrs and after 72 hrs of life respectively. Difference in NLR between both the groups was assessed.

Statistical Analysis: Data was entered in Microsoft Excel and analyzed with SPSS version 17.0. Categorical variable like gender was expressed as proportions. NLR was derived from ALC and ANC. Continuous parameters like TLC, ALC, ANC and NLR values between the cases and controls were compared using Independent t test or student t test and mean (SD) was reported. Comparison of gender between the cases and controls was done with hi squared test. One way ANOVA was used to compare the difference in TLC, ALC, ANC and NLR values across the culture positive, screen positive and clinical sepsis cases. Independent t test was used to compare the difference in TLC, ALC, ANC and NLR values across EOS and LOS categories. Receiver operating characteristics curve was used to derive the cut off of NLR to predict the culture positive cases and sensitivity, specificity, PPV, NPV and diagnostic accuracy values were reported along with its 95% confidence limits. A p<0.05 was considered as statistically significant.

RESULTS AND DISCUSSIONS

Demographic details are homogenous in both cases and controls with respect to birth weight, gestational age and gender with no statistically

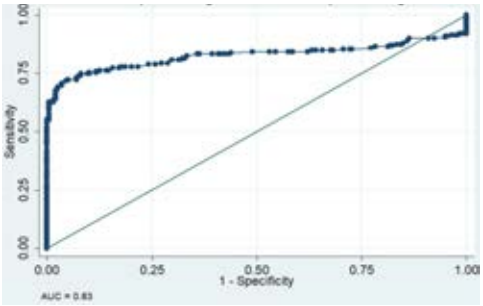

Fig.1: ROC for predicting the neonatal sepsis using NLR

Table-1: Comparison of baseline characteristics across the study participants

	Cases		Controls		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Gestational age	36.5	2.8	36.8	2.8	0.28
Birth weight	2.4	1.3	2.5	0.6	0.27
Gender, n(%)	95	48.2	91	46.0	0.66
FemaleMale	103	51.8	107	54.0	

Table-2: Comparison of hematological parameters across the study participants

	Cases		Controls		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**
TLC	11515	6798.2	10335.4	4535.8	0.04
ANC	7431.4	5110.6	5642.5	2582.6	<0.001
ALC	3126.7	1757.6	4248.8	1944.4	<0.001
NLR	2.6	1.5	1.3	0.2	<0.001

Table-3: Comparison of hematological parameters between different groups

Parameter	**Culture positive**		**Screen positive**		**Clinical sepsis**		
	sepsis(n = 84)		**sepsis(n = 125)**		**(n=47)**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
TLC	12051	4172.2	11959.7	8112.0	9625.4	6769.9	0.12
ANC	7993.6	3825.7	7768.5	5953.7	6087.9	5135.2	0.13
ALC	3326.2	1372.9	3097.2	1739.7	2684.5	1814.6	0.20
NLR	2.6	1.2	2.7	1.9	2.4	1.3	0.71

Table 4: Comparison of hematological parameters between EOS and LOS

Parameter	**EOS(n = 128)**		**LOS(n = 70)**		
	Mean	**SD**	**Mean**	**SD**	**p-value**
TLC	12201.3	7173.6	10261.1	5896.1	0.04
ANC	7919.7	5320.6	6538.4	4605.5	0.05
ALC	3275.5	1910.2	2854.6	1409.8	0.08
NLR	2.7	1.6	2.4	1.2	0.17

Table 5: Statistical parameters for NLR

Parameter	**Point estimate**	**95 % Confidence limit**
Sensitivity	78.8	72.4-84.3
Specificity	75.8	69.2-81.6
Positive predictive value	76.5	70.0-82.1
Negative predictive value	78.1	71.6-83.8
Diagnostic accuracy	77.3	72.8-81.3

Table 6:Comparison of hematological parameters between different studies

	Sumitro,K.R., *et al.*[14]	**Li,T., *et al.*[18]**	**Panda,S.K., *et al.*[15]**	**Lim,H., *et al.*[17]**	**Present study**
Place of study	Dr.Soetomo hospital, Indonesia	Henan children's hospital, China	Kalinga institute of medical sciences, Bhubaneshwar,	Sanglah hospital , Indonesia	SIMS, Shimoga, Karnataka India
Study period	April to	January 2016 to December 2019	January 2019 to January 2020	April 2018 to september2018	June 2022 to Dec 2022
TLC	11265	9990	12230+/-6752		11515+/-6782.2
ANC	6375	5070	8229+/-5456	10250	7431+/-5110.6
ALC	2010	3340	2795+/-1424	3430	3126+/-1757.6
NLR		1.65	3.88+/-1.78	2.94	2.6+/-1.5

Table 7:Comparison of statistical parameters between different studies

	Sumitro,K.R., *et al.*[14]	**Li,T., *et al.*[18]**	**Mahmoud,N.M.S.A., *et al.*[16]**	**Panda,S.K., *et al.*[15]**	**Lim,H., *et al.*[17]**	**Present study**
Place of study	Dr.Soetomo hospital, Indonesia	Henan children's hospital, China	Minia university children, Egypt Bhubaneshwar, India	Kalinga institute of medical sciences,	Sanglah hospital , Indonesia,	SIM, Shimoga Karnataka India
Study period	April to september 2019	January 2016 to December 2019	July 2018 to January 2019	January 2019 to January 2020	April 2018 to	June 2022 September 2018to Dec 2022
Cut off	2.12	1.62	0.1	1.7	2.31	1.5
Sensitivity	80.4	51	67	68.3	26.1	78.8
Specificty	42.3	75	99	46.2	81.8	75.8
PPV	58.3		98	50	66.1	76.5
NPV	68.8		75	64.86	91.1	78.1

significant difference i.e p>0.05.(Table 1) On comparing cases with controls, no statistical significant difference in noted with respect to mean total leucocyte count. But there is statistical significant (p <0.001) difference is noted with respect to mean of absolute neutrophil count and absolute lymphocyte count. Similarly Statistical significant difference is noted in neutrophil lymphocyte ratio (NLR) with p<0.001 between cases and controls.(Table 2) In the study groups, cases were divided into three groups, culture positive, only screen positive and both culture and screen negative. Out of 198 cases 84 were culture positive, 125 cases were screen positive and 47 were both culture and screen negative i.e., clinical sepsis. On comparing between these three groups, no statistical significant difference is noted with respect to mean TLC, Mean ANC, mean ALC and NLR.(Table 3)

In this group, 128 cases were early onset sepsis (EOS) and 70 cases were late onset sepsis (LOS). On comparing these two groups, no statistical significant difference is noted with respect to mean TLC, Mean ANC, mean ALC and NLR.(Table 4) The NLR cut off of >1.5 had sensitivity of 78.8%, specificity of 75.8%, positive predictive value(PPV) of 76.5% and negative predictive value of 78.1% .(Table 5). The area under curve (AUC) for NLR in diagnosing neonatal sepsis was 0.83.(Fig 1)

Diagnosis Area under the curve 95 % CI for AUC P value Exudate 0.83 0.78-0.87 <0.001 Neonatal sepsis is a life threatening disease, early detection helps in

preventing mortality and morbidity. Currently blood culture and sepsis screen are in use to detect sepsis, but blood culture is less sensitive and takes longer duration in detecting sepsis. Sepsis screen has variable sensitivity and specificity. Recently NLR has attracted substantial attention as a new risk factor in diagnosing sepsis. Total leucocyte count and lymphocyte count can be easily available while doing CBC using autoanalyser.

During sepsis dynamic changes occurs in neutrophils and lymphocytes. Neutrophils are the first line of defense after the invasion of microbes. The primary role of neutrophils is to fight against bacteria and stimulates the process of phagocytosis. The life span of neutrophils increases due to decrease in caspase 3 levels and NF-KB activation[10,11]. Lymphopenia is seen in sepsis due to migration of lymphocytes to the site of infection[12,13]. This study includes 198 cases and 198 controls. The study group was larger than the studies conducted by Sumitro, K. R., *et al.*[14], Panda, S. K., *et al.*[15], Mahmoud, N. M .S .A., *et al.*[16], Lim, H., *et al.*[17]. Out of 198 cases 103 were males and 95 were females.

This study had TLC of 11515+/-6782.2, ANC of 7431+/-5110.6 and ALC of 3126/-1757.6 which is comparable with the other studies conducted by Sumitro, K. R., *et al.*[14], Li,T., *et al.*[18], Panda, S. K., *et al.*[15] and Lim, H., *et al.*[17]. NLR of 2.6+/-1.5 which was higher compared to study done by Li,T., *et al.*[18] and lower compared to Panda, S. K., *et al.*[15] and Lim, H., *et al.*[17].(Table 6) Cases were divided into three groups as culture positive, screen positive and clinical sepsis groups. No statistical difference is noted in NLR between these three groups. But in contrast significant difference is noted in NLR between culture positive and culture negative cases in study conducted by Sumitro, K. R., *et al.*[14].

In this group, 128 cases were early onset sepsis (EOS) and 70 cases were late onset sepsis (LOS). On comparing these two groups, no statistical significant difference is noted with respect to NLR. But in contrast study conducted by Sumitro,K.R., *et al.*[14] noted statistical significant difference is noted in NLR between EOS and LOS. In this study neonates with sepsis had NLR of 2.6+/-1.5 had area under curve of 0.83with cut off of 1.5. Similar cut off is seen in studies conducted by Li,T., *et al.*[18] and Panda, S. K., *et al.*[15] and higher cut off was seen in Sumitro, K. R., *et al*[14]. But study conducted by Mahmoud, N.M.S.A., *et al.*[16] has very low cut off of 0.1. Sensitivity of NLR is 78.8% in the present study , similar findings were noted in studies conducted by Sumitro,K.R., *et al.*[14], Mahmoud,N.M.S.A., *et al.*[16] and Panda,S.K., *et al.*[15]. But lower sensitivity was seen in studies conducted by Li,T., *et al.*[18] and Lim,H., *et al.*[17]. In this study NLR had specificity of 75.8%, which is comparable with Li,T., *et al.*[18] and Lim,H., *et al.*[17]. But lower specificity is seen in Sumitro,K.R., *et al.*[14] and Panda,S.K., *et al.*[15]. (Table 7)

Limitation: Since this is cross sectional study follow up of neonates for change in NLR values after starting treatment was not done , hence dynamic correlation between sepsis and NLR values cannot be assessed. Other parameters like type of organism and prematurity affecting NLR values were not done in the study.

CONCLUSION

This study demonstrated strong correlation between neutrophil-lymphocyte ratio and neonatal sepsis with sensitivity and specificity of 78.8% and 75.8% respectively. Further studies with larger sample size are necessary to strongly conclude this association. To conclude, NLR is cheaper and widely available markers in diagnosing neonatal sepsis in developing counties with resource limited setting.

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