

Neutrophil-lymphocyte Count Ratio as an Indicator of Culture Positive versus Culture Negative Sepsis: A Single-centre Cross-sectional Study

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ABSTRACT

Introduction: In sepsis patients, the Neutrophil-lymphocyte Count Ratio (NLCR) is a laboratory statistic that can indicate bacterial infection.

Aim: To measure NLCR in patients with sepsis and compare the NLCR in patient with culture positive and culture negative sepsis.

Materials and Methods: This single-centre, cross-sectional study was conducted in the Department of General Medicine at R.L. Jalappa Hospital (Sri Devaraj URS Medical College), Kolar, Karnataka, India, from October 2021 to December 2021. A total of 120 patients above 18 years of age with sepsis, diagnosed as per the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), were included in the study. Participants were evaluated for sepsis and septic shock (society of critical care medicine conference definitions) the principles of initial resuscitation (fluid therapy, vasopressors, inotropic support), and infection issues (source identification and control, appropriate antibiotic therapy) were followed regularly and the

outcome studied. Neutrophil count, lymphocyte count, and calculation of NLCR were done at the time of admission. The correlation studies of NLCR in culture-positive and culture-negative was done and compared. Data was analysed by using coGuide software (version 1.03).

Results: The mean age of patients was 58.98±17.78 years, ranged from 20 to 96 years, 47 (39.17%) were male and 73 (60.83%) were female, majority 93 (77.50%) out of 120 patients had fever. Majority 78 (65%) had type 2 diabetes mellitus. Neutrophil-to-Lymphocyte ratio (NLR) was 10±1.67, ranging from 7.40 to 14.50. The area under the ROC curve was 0.522. There was no statistically significant relationship between the NLCR and culture report (p-value=0.216). The NLCR had poor predictive validity in predicting culture positive, as indicated by the area under the curve {0.522 (95% CI: 0.417 to 0.626, p-value= 0.988)}.

Conclusion: NLCR can be considered as predictor for the initiation of treatment of patients with sepsis.

Keywords: Inotropic support, Resuscitation, Septic shock, Vital signs

INTRODUCTION

Sepsis and septic shock have an 85% morbidity and death rate, making it a public health issue [1]. There were 4711 admissions in an Indian tertiary care hospital throughout the course of a five year prospective observational research on severe sepsis, with 282 (6.2%, 95% Confidence Range: 2.3- 13.1) having severe sepsis. The Intensive Care Unit (ICU) death rate was 56%, the hospital mortality rate was 63.6%, and the 28 day mortality rate was 62.8% [2].

Severe sepsis was widespread in Indian Intensive Treatment Units (ITUs), according to a multicentre, prospective, observational study done in four ITUs in India. In comparison to the western literature, ITU mortality was greater [3]. Bacteria are by far the most prevalent causal microorganisms in sepsis, with positive cultures occurring in roughly half of the cases. Failure to use antibiotics that the microorganisms are sensitive to is linked to a higher risk of death [4,5].

Neutrophils make up 50 to 70% of all circulating leukocytes in humans, and they constitute the first line of defence against a variety of infectious diseases such as bacteria, fungus, and protozoa. Bacteria are by far the most common cause of sepsis, with positive cultures appearing in nearly half of the cases. Failure to utilise antibiotics that are sensitive to the bacteria is connected to a greater risk of mortality. As a result, between 55% and 60% of bone marrow is committed to their creation [6,7]. Neutrophils are a significant arm of the innate immune system, armed with a range of weapons against infections. Because of their effect on immunological responses to pathogenic microbes and other foreign substances, lymphocytes play a critical function in the immune system. Absolute neutrophil

(immature granules, rods, and segments) and absolute lymphocyte values based on the number of leukocytes are used to calculate the Neutrophil-Lymphocyte Count Ratio (NLCR) [5]. The Neutrophil-Lymphocyte Ratio (NLR) is a well-known inflammatory measure that reflects systemic inflammatory response, infectious diseases, and surgical sequelae [8-10].

Blood cell analysis and the NLR, together with other diagnostic tests, were revealed to be predictors for the severity of gram negative sepsis by Gharebaghi N et al., [11]. According to the findings of a meta-analysis research, NLR may be a useful predictive biomarker for patients with sepsis, and greater NLR values may suggest a worse prognosis in these individuals [12]. Hence, this study was conducted to measure NLCR in patients with sepsis and compare the NLCR in patient with culture positive and culture negative sepsis.

MATERIALS AND METHODS

This single-centre, cross-sectional study was conducted in the Department of General Medicine at R.L. Jalappa Hospital (Sri Devaraj URS Medical College), Kolar, Karnataka, India, from October 2021 to December 2021. The study was approved by the Institutional Human Ethics Committee and Institutional Review Board (Reference DMC/KLR/IEC/308/2021-22). Data confidentiality was maintained. Written informed consent was obtained from the patients.

Inclusion criteria: A total of 120 Patients of above 18 years of age with sepsis {diagnosed as per Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)} [13] were included in the study.

Exclusion criteria: Patients with pre-existing organ dysfunction prior to infection (chronic kidney disease, decompensated liver disease, cardiac disease), blood product transfusion in the week before admission, patients with haematological diseases such as, hypersplenism, haematological malignancy, metastatic bone marrow infiltration by malignancy, recovery after bone marrow hyperplasia and those with recent chemotherapy as well as pregnant women were subjected for exclusion from the study.

Procedure

Clinical, and laboratory data was obtained and studied before administration of broad-spectrum antibiotics. Study participants were assessed for sepsis and septic shock (society of critical care medicine conference definitions) [13]. The principles of initial resuscitation (fluid therapy, vasopressors, inotropic support) and infection issues (source identification and control, appropriate antibiotic therapy) were followed regularly and the outcome studied. Investigations like complete blood count, Electrocardiogram (ECG), chest X-ray, blood culture, urine culture, Endotracheal tip Test (ET) culture, sputum culture were carried out on all the study participants. Neutrophil count, lymphocyte count and calculation of NLCR were done at the time of admission. The correlation studies of NLCR in culture positive and culture negative was done and compared.

STATISTICAL ANALYSIS

Categorical data like gender, presenting illness etc. is represented in the form of frequencies and proportions. Continuous data like age, temperature (Fahrenheit), pulse (beats per minutes), haemoglobin (g/dL), mean platelet volume and red blood cell distribution width was represented as mean and standard deviation. The count variables are analysed by the Chi-square test expressing as proportion. The utility of neutrophil lymphocyte ratio in predicting culture report was assessed by Receiver Operative Curve (ROC) analysis. Area under the ROC curve along with its 95% CI and p-value are presented. Basing on the ROC analysis, it was decided to consider 9.50 as the cut-off value. The sensitivity, specificity, predictive values and diagnostic accuracy of the screening test with the 95% CI are presented. A p-value <0.05 was considered statistically significant. Data was analysed by using coGudie software (version 1.0.3) [14].

RESULTS

A total of 120 subjects were included in the final analysis. The mean age was 58.98±17.78 years, ranged from 20 to 96 years, 47 (39.17%) were male and 73 (60.83%) were female, majority 93 (77.50%) out of 120 patients had fever. Majority 78 (65%) had type 2 diabetes mellitus [Table/Fig-1].

Parameters	n, %
Gender	
Male	47 (39.17%)
Female	73 (60.83%)
History of presenting illness	
Fever	93 (77.50%)
Cough	54 (45%)
Breathlessness	43 (35.83%)
Altered sensorium	39 (32.50%)
Vomiting	13 (10.83%)
Headache	3 (2.5%)
Decreased urine	1 (0.83%)
Others	37 (30.83%)
Medical history	
T2 diabetes mellitus	78 (65%)
Hypertension	46 (38.33%)
Hypothyroidism	12 (10%)

Coronary artery disease	9 (7.50%)
Chronic obstructive pulmonary disease	8 (6.67%)
Bronchial Asthma	6 (5%)
Ischaemic heart disease	3 (2.5%)
Epilepsy	2 (1.67%)
Pulmonary tuberculosis	1 (0.83%)

[Table/Fig-1]: Summary of baseline parameter (N=120).

The mean body temperature was 99.98±1.33 °F, ranged between 98 to 102 °F, the mean pulse was 106.23±15.79 beats per minute, ranged between 60 to 140, 2 (40.00%) had pallor, 6 (5.08%) had hepatomegaly, 3 (2.59%) had splenomegaly, 98 (83.76%) had reported normal urine routine, the mean haemoglobin was 13.71±1.43 (g/dL) ranged from 11.20 to 17.50 g/dL. The NLCR was 10±1.67, ranged from 7.40 to 14.50; the mean platelet volume was 9.25±1.07, ranged from 7.50 to 11.40; and mean red blood cell distribution width was 15±1.11%, ranged from 13 to 17.80%. The mean Glasgow Coma Scale (GCS) score from day 1 to 5 increased gradually [Table/Fig-2].

Parameters	Results
Vital signs	
Temperature °F	99.98±1.33
Pulse (beats per minute)	106.23±15.79 (range 60 to 140)
Pallor and cyanosis (N=5)	
Pallor	3 (60%)
Cyanosis	2 (40%)
Hepatomegaly (N=118)	
Splenomegaly (N=116)	
Urine routine (N=117)	
Normal	98 (83.76%)
Pus cells	13 (11.11%)
Protein	6 (5.13%)
Laboratory parameter	
Haemoglobin (g/dL)	13.71±1.43 (range 11.20 to 17.50)
Neutrophil-to-lymphocyte ratio	10±1.67 (range 7.40 to 14.50)
Mean platelet volume (fL)	9.25±1.07 (range 7.50 to 11.40)
Red blood cell distribution width (%)	15±1.11 (range 13 to 17.80)
Glasgow coma scale scores	
Day 1	12.95±2.09 (range 1 to 15)
Day 2 (N=112)	12.9±2.23 (range 1 to 15)
Day 3 (N=100)	13.42 ± 2.14 (range 1 to 15)
Day 4 (N=92)	13.7 ± 2.11 (range 1 to 15)
Day 5 (N=89)	14.01 ± 2.17 (range 0 to 15)

[Table/Fig-2]: Summary of vital sign and lab parameter (N=120).

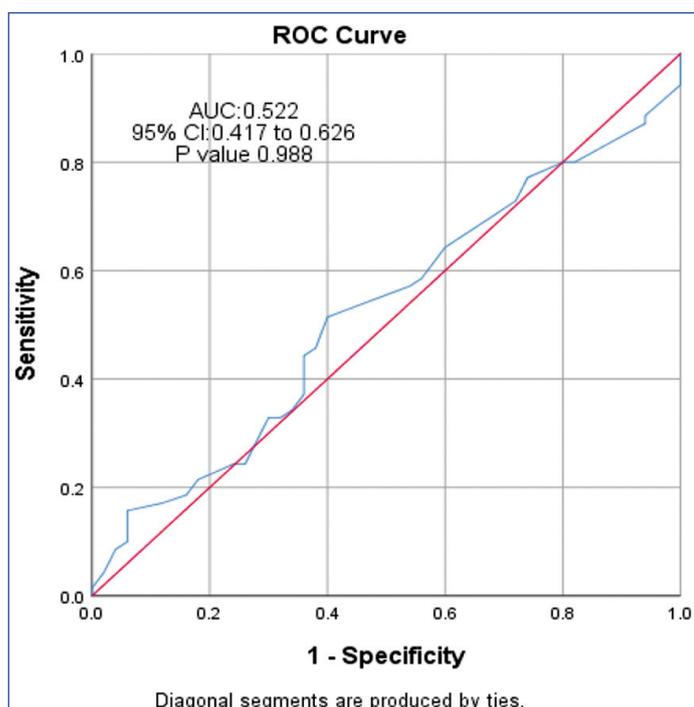
Among the study population, more than half in proportion required ventilation support and inotropic support, while few required renal replacement. The mean ICU stay 3.41 days, but the survival rate was only 65.83% (79 out of 120). The mean SOFA score at day 1 to day 5 was decreasing in trend from almost 6.52 to 4.04. Major diagnosis was LRTI followed by urosepsis and ARS [Table/Fig-3].

Parameters	Results
Ventilator support	69 (57.5%)
Inotropic support (N=119)	80 (67.23%)
Renal replacement (N=115)	15 (13.04%)
Duration of ICU Stay (in days)	3.41±1.98 (range 1 to 12)
Survived	79 (65.83%)
SOFA Score	
Day 1	6.52±3.36 (range 1 to 16)
Day 2 (N=112)	6.83±3.7 (range 1 to 17)

Day 3 (N=100)	5.87 ± 3.86 (range 0 to 18)
Day 4 (N=93)	4.74 ± 3.37 (range 0 to 16)
Day 5 (N=90)	4.04 ± 3.43 (range 0 to 17)
Diagnosis	
Lower Respiratory Tract Infections (LRTI)	76 (63.33%)
Urosepsis	19 (15.83%)
Acute Respiratory Distress Syndrome (ARDS)	15 (12.50%)
Meningitis	9 (7.50%)
Cellulitis	8 (6.67%)
Acute Gastroenteritis	7 (5.83%)
Neurological infections	1 (0.83%)

[Table/Fig-3]: Summary of other parameters (N=120).

The NLCR had poor predictive validity in predicting culture positive, as indicated by area under the curve of 0.522 (95% CI: 0.417 to 0.626, p-value=0.988) [Table/Fig-4].



[Table/Fig-4]: Predictive validity of Neutrophil lymphocyte ratio in predicting positive culture report (ROC analysis).

Out of 70 participants with culture positive, the NLCR was high (≥ 9.50) for 36 (51.43%) and low (< 9.50) for 34 (48.57%). There was no statistically significant relationship between culture report and NLCR (p-value=0.216). The ratio had a sensitivity of 51.43% (95% CI: 39.17% to 63.56%) in predicting culture positive, specificity was 60.00% (95% CI: 45.18% to 73.59%), false positive rate was 40% (95% CI: 26.41% to 54.82%), false negative rate was 48.57% (95% CI: 36.44% to 60.83%), positive predictive value was 64.29% (95% CI: 50.36% to 76.64%), negative predictive value was 46.88% (95% CI: 34.28% to 59.77%), and the total diagnostic accuracy was 55.00% (95% CI: 45.65% to 64.09%) [Table/Fig-5].

NLR	Culture report		χ^2 value	p-value
	Positive (N=70)	Negative (N=50)		
High (≥ 9.50)	36 (51.43%)	20 (40%)	1.531	0.216
Low (< 9.50)	34 (48.57%)	30 (60%)		
Parameter	Value		95% CI (Lower - upper)	
Sensitivity	51.43%		39.17%	63.56%
Specificity	60.00%		45.18%	73.59%
False positive rate	40.00%		26.41%	54.82%

False negative rate	48.57%	36.44%	60.83%
Positive predictive value	64.29%	50.36%	76.64%
Negative predictive value	46.88%	34.28%	59.77%
Diagnostic accuracy	55.00%	45.65%	64.09%

[Table/Fig-5]: Predictive validity of NLR in predicting culture report (N=120).

DISCUSSION

The finding of the present study represents that NLCR was higher in the culture positive patients when compared with culture negative patients and NLCR was 1.67, ranged from 7.40 to 14.50. The NLCR was substantially greater (p-value=0.001) in the severe sepsis group (median = 21.1 with quartiles=11.1 to 42.4) compared to the group without severe sepsis (median = 11.6 with quartiles=7.6 to 18.9) in a study by Ljungstrom L et al., [15]. Sen V et al., found that the incidence of sepsis was significantly higher in patients with NLCR ≥ 2.50 than in patients with NLCR < 2.50 (p-value=0.006) [16].

The results of current research demonstrate that sensitivity of NLCR was 51.43% with specificity of 60%. With the help of the present study data, the NLCR specificity for sepsis diagnosis was higher could be related. We found that NLCR had positive predictive value of 64.29% and diagnostic accuracy of 55%. Orfanu A et al., estimated the optimal cut-off value of NLCR at 8.18, with a sensitivity of 70.5% and a specificity of 72.2% [17]. These findings were similar to our results. In another study by Hota PK and Reddy BG, NLR was found to have 86.2% sensitivity, 85.7% specificity, positive predictive value of 89.2%, negative predictive value of 81.1% in predicting diagnosis and prognosis of sepsis [18]. Their results showed higher sensitivity than specificity but with minor difference.

Similarly, another study by Mandal RK and Valenzuela PB, reported that sensitivity and specificity of NLCR was 97.37% and 93.18, respectively [19]. In a meta-analysis the pooled analyses from eight studies results depicted that the diagnostic accuracy of the NLCR in terms of its bacteraemia-sensitivity was 0.723, and specificity was 0.596. The area under the summary receiver operating characteristic curve was 0.69 [20].

In this study, there were majority of females diagnosed with sepsis as compared to males. Pietropaoli AP et al., also found that among 18,757 ICU patients (median age, 66 years; interquartile range, 53-77 years), 8702 were females (46%). Female patients had a greater hospital mortality rate than male patients (35% versus 33%, respectively; p-value=0.006). After accounting for differences in baseline characteristics and care procedures, they discovered that females were more likely than men to die in hospitals (odds ratio=1.11; 95% CI: 1.04-1.19; p-value=0.002) [21]. There were 2,345 (64.37 %) male and 1,298 (35.63 %) female patients in the study by Zhou X et al., they also reported that female patients with septic shock had a greater in-hospital death rate (55.54% vs. 49.29%, p-value=0.01) than male patients [22]. Majority of the study population suffered from fever. However, a clinical review suggest that fever is a cornerstone diagnostic sign in clinical practice that helps to start early appropriate therapy and to follow the infection course [23]. Hence, it can be concluded that high NLCR score can be considered for initiation of treatment of patients with sepsis.

Limitation(s)

The sample size was limited. Although blood culture-positive and culture-negative groups were similar in terms of age and gender, there may have been other important differences between them. For example, information regarding diagnostic group, co-morbidities and discharge status was not available.

CONCLUSION(S)

In the present study, NLCR ranged from 7.40 to 14.50 and showed a high positive predictive value. Out of the 70 culture positive participants, 36 (51.43%) had a high NLCR. NLCR is simple, easily measured, reacts very early in the course of acute inflammation and

easy to use in daily practice without extra costs compared to blood culture report which was considered as gold standard.

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