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Original Research Article

SIGNIFICANCE OF NEUTROPHIL TO LYMPHOCYTE RATIO IN COVID- 19 PATIENTS : IN A TERTIARY CARE HOSPITAL

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ABSTRACT

The study was conducted to assess the association of neutrophil lymphocyte ratio (NLR) in COVID- 19 and to identify the cut-off value that predicts mortality, need of respiratory support and admission to high-dependency or intensive care.

METHODS: A retrospective observational study was conducted to collect demographic data, clinical variables, the neutrophil-lymphocyte ratio on-admission and the outcome of confirmed COVID-19 patients admitted to a tertiary care centre.

OBJECTIVE: To determine the significance of Neutrophil to Lymphocyte ratio (NLR) in Covid- 19 patients.

RESULTS: Of the 150 patients as sample size in this study, the median Neutrophil count was $4.07 \times 10^3/\mu L$ (IQR 2.97-6.79) and the median Lymphocyte count was $1.74 \times 10^3/\mu L$ (IQR 1.36-4.75). The calculated NLR ranged from 0.12 to 48.28 with a median value of 2.32 (IQR 1.37-4.76). An NLR value >3.6 predicted development of severe disease requiring respiratory support, transfer to a high-dependency or an intensive care unit and/or succumbing to the illness with a sensitivity 80% and specificity 80% (area under the curve 0.8, 95% CI 0.72-0.88, $P < .0001$). The adjusted odds ratio of NLR > 3.6 on predicting severe disease was 11.1, 95% CI 4.5- 27.0, $P < .0001$.

CONCLUSIONS: A NLR > 3.6 is a useful variable to be included in risk prediction scores

KEYWORDS: COVID-19, neutrophil lymphocyte ratio, NLR, severe disease, SARS-CoV-2

INTRODUCTION:

The 2019 coronavirus disease (COVID-19) was first identified in December 2019 in Wuhan, China caused by the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), commonly known as the COVID-19 virus (1). Since then, the virus has spread globally, resulting in an on-going pandemic. As of June 4, 2020, over 6 million cases have been reported across 216 countries and territories, resulting in more than 382,000 deaths. As of June 4, 2020, over 6 million cases have been reported across 216 countries and territories, resulting in more than 382,000 deaths (2). It has rapidly and widely spread worldwide, and was declared a pandemic in March 2020 by World Health Organization (2,3).COVID- 19 is a member of the genus Betacoronavirus.1–4 Infection of COVID-19 can trigger and lead to overproduction of pro-inflammatory cytokines and chemokines including interleukin-1β (IL-1β), interleukin-6

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(IL-6), and tumor necrosis factor-α (TNF-α) by the immune system, resulting in multiple organ injury (4).Increased plasma levels of inflammatory cytokine have been found in patients with COVID-19. Various biochemical and hematological parameters are deranged. One of the study shows the neutrophil-lymphocyte ratio (NLR) to serve as a reliable indicator of severe COVID-19. Additionally, critically ill COVID-19 patients show higher NLR when compared with non-ICU patients [5]. Xia et al., 2020 [6] found that approximately 80% of SARS-CoV-2 patients infected with bilateral pulmonary involvement have increased NLR (7).This study is aimed to determine the role of Neutrophil to Lymphocyte ratio (NLR) in Covid- 19 patients in a tertiary care hospital.

OBJECTIVE:

To determine the significance of Neutrophil to lymphocyte ratio (NLR) in Covid- 19 patients.

MATERIALS AND METHODS:

This study was conducted at Department of Pathology, ESIC Medical College & Hospital, Kalaburagi.

Study population: All patients admitted in ESIC Medical College and Hospital, Kalaburagi from April 2021 to June 2021

Sample size: 150 samples

Inclusion criteria: All hospitalized patients over 18 years old with confirmed COVID-19 infection by RAT or RTP-PCR during study period at ESIC Medical College & Hospital, Kalaburagi.

Exclusion criteria: Patients who are COVID-19 negative by RT-PCR , patients with inconclusive RT-PCR test report and with other comorbidities such as cancer, hematological diseases, severe cardiac disease (NYHA III and IV cardiac failure, recent myocardial infarction -last three months, unstable arrhythmia), liver disease, systemic diseases, and pulmonary fibrosis and any other suspected or confirmed Co- infections.

Data collection: We retrospectively evaluated and analyzed medical history, physical examination, need for intubation and intensive care unit follow up and mortalities from inpatient department files from medical records department (MRD) and complete blood investigations will be obtained from laboratory records. Then according to Government of India Ministry of Health and Family Welfare's guidelines patients will be divided into mild, moderate and severe cases. Demographic, clinical, laboratory, and treatment data will be taken from the patients at admission and extracted from electronic medical records. Laboratory tests will be collected from all the patients at entry in the hospital (before any intervention) and will be recorded.

Neutrophil (NEU)-to-lymphocyte (LYM) ratio (NLR) will be calculated as the absolute count of Neutrophils divided by the total count of lymphocytes. Blood examinations involved measuring complete blood cell count and differential Values. The study will include all patients with confirmed SARS-COV 2 infection consecutively hospitalized during study period. COVID-19 diagnostics were confirmed using real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay to test nasal and pharyngeal swab specimens according to WHO guidance.

Statistical Analysis: Statistical analysis was carried out using statistical package for social science (SPSS) Version 20.0. The Wilcoxon rank sum tests were applied to continuous variables, and chi-square and Fisher's exact tests were used for categorical variables. The optimal cut-off values of the continuous neutrophil (NEU)-to-lymphocyte (LYM) ratio (NLR),

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was calculated by applying the receiver operating curve (ROC) analysis. Hazard risk (HR) and 95% confidence interval (CI) were used as common measures to assess relative risk. $P < 0.05$ was recognized as statistically significant. The optimal cut-off values was 3.3
The laboratory reference values of White blood cells, Neutrophils, Lymphocytes, Eosinophils and Monocytes were 4.2–10, 1.8–7.3, 1.5–4, 0.05–0.35 /uL and 0.1–0.6/uL, respectively.

RESULTS

There were 150 COVID-19 confirmed patients included inthe study and moderate-severe in 50 (24%) patients. There were 26 (12.5%) patients requiring non-invasive ventilation, high-flow nasal oxygen or invasive ventilation and 39 (18.8%) required high-dependency or intensive care unit admission for organ support. There were 37 (17.8%) deaths in the study group. Patients who deteriorated in the hospital requiring respiratory support, transfer to a high-dependency unit (HDU) or an intensive care unit (ICU) and patients who succumbed to the illness were classified as the "severe disease" group. A significantly higher heart rate (100.5 vs. 91, $P < .0001$) and a lower oxygen saturation (SpO_2) (88.5 vs. 98, $P < .0001$) was seen on-admission to hospital in patients who developed severe disease compared to non-severe category (Supplemental Table 1). Investigations revealed a higher C-reactive protein (CRP) (134.4 vs 20.8, $P < .0001$), a higher white cell count ($9.3 \times 10^3/\mu L$ vs. $6.7 \times 10^3/\mu L$, $P < .0001$), higher serum glutamic-oxaloacetic transaminase (SGOT) (56.5 vs. 35.3, $P < .01$) and higher serum creatinine (141.9 vs. 79, $P < .0001$) on-admission in patients who developed severe disease com- pared to others. The median neutrophil count was $4.07 \times 10^3/\mu L$ (IQR 2.97-6.79) and the median lymphocyte count was $1.74 \times 10^3/\mu L$ (IQR 1.36-4.75) in the study population. The calculated NLR ranged from 0.12 to 48.28 with a median value of 2.32 (IQR 1.37-4.76). A higher absolute neutrophil, a lower absolute lymphocyte count and a higher NLR were significantly associatedwith the need for respiratory support in the form of non-invasive ventilation, high-flow nasal oxygen or invasive ventilation. In addition, a higher neutrophil count, a lower lymphocyte count and a higher NLR was significantly associated with the need of escalation to a high-dependency unit, intensive care unit and associated with mortality. Our data revealed that the NLR on-admission to the hospitalin patients with COVID-19 predicted severe disease and in- hospital deterioration. We developed ROC curve for further identification of the association of NLR with severe disease. A NLR value higher than 3.6 was the best cut-off value identified to predict severe disease with a sensitivity of 80% and a specificity of 80%. Since the area under the curve (AUC) for the selected cut off for NLR >3.6 was 0.8 (95% CI 0.72-0.88, $P < .0001$), respective NLR value on admission canbe used as an excellent discriminator in identifying the severe disease outcome in Sri Lankan population. Patients with NLR >3.6 were older and had a higher heartrate and a lower SpO_2 on-admission. The CRP,SGOT and creatinine were higher among patients with aNLR > 3.6 compared to patients with a value $\leqslant$ 3.6.The odds ratio NLR > 3.6 on predicting severe disease was 15.4 (CI 6.5-36.5, $P < .0001$) and the risk persisted after correcting for the possible confounding effect of age (adjusted OR11.1, CI 4.5-27.0, $P < .0001$)

Table 1 Characteristics of patients presenting with Covid-19 infection.

	COVID19	P value
N	150	
Age (years)[a]	67 (28)	P < 0.001

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	COVID19	P value
Sex (% males)	57.6	P < 0.001
Charlson comorbidity index[a]	3 (4)	P < 0.001
Body temperature[a] (°C)	37.4 (1.2)	P < 0.001
Oxygen saturation[a] (%)	96 (5)	P = 0.074

Table 2 Inflammatory markers of patients presenting with Covid-19 infection.

	COVID19	P value
Lymphocytes (10e3/µL)[a]	1.0 (0.8)	P < 0.001
Neutrophils (10e3/µL)[a]	4.9 (3.9)	P < 0.001
NLR[a]	4.5 (5.2)	P < 0.001
CRP (mg/L)[a]	57.3 (115.3)	P = 0.98
Cratinine	434.4 (746)	P< 0.001
SGOT[a]	123 (80.3)	P < 0.001

DISCUSSION

Cheap and widely available markers are useful in triaging patients with COVID-19 at community level and in healthcare settings. We report the association of severe COVID-19 and the NLR performed on-admission to a tertiary care. We have derived the best cut-off value of NLR to be used to screen patients who are at high risk of deterioration at first presentation during the COVID-19 pandemic. The age distribution and the presence of comorbidities in our study population were comparable to studies from the region.[8,9] Increasing age and male gender is known to be associated with severe disease.[5] Previous studies have revealed poor T-cell activation, higher ACE-2 levels and poor habits such as smoking contributing to a worse outcome in males compared to females.[10-12] Although not statistically significant, a higher frequency of diabetes and hypertension was seen in the severeCOVID-19 category of our study. The lower numbers in the groups could have affected the power in identifying a significant association. The presence of tachycardia on-admission and a high CRP correlated with a severe disease outcome and both these parameters were associated with a high NLR suggesting that these patients had features of a systemic inflammatory response Neutrophils are components of the first line of defense in infections and they are involved in the inflammatory response to injury. A high neutrophil count initiates the systemic inflammation seen during the cytokine mediated response in COVID-19. Lymphocytes are important for initiating the immune response to infection and low lymphocytes have shown to be associated with a severe disease outcome.[13,14] NLR which incorporates both neutrophil andlymphocyte counts is therefore, a useful prognostic markerin COVID-19. Despite the available literature on the association of NLRas a marker of disease severity in COVID-19, cut-off values identified are highly variable suggesting the heterogeneous distribution of this parameter. The NLR value considered normal for a healthy adult is not clear. Forget et al, reporteda mean NLR of 1.65 in a Belgian

population of apparentgood health and the upper limit was reported as 3.5.[15,16] There are no data available in other regions. Studies conducted in COVID-19 patients in Asia report cut-off values ranging from 4 to 11 in predicting mortality and severe disease.[16] The presence of metabolic syndrome, non-alcoholic fatty liver disease and diabetes affect the baseline NLR of an individual. It is important to identify the population-specific NLR cut-off values in countries with a high prevalence ofsuch metabolic conditions as Sri Lanka. Our data reveal thata NLR more than 3.6 had a sensitivity and specificity of 80% in identifying patients likely to develop severe COVID-19.A patient having a NLR>3.6 at first presentation had 11-times higher risk of dying, needing respiratory support or requiring HDU or ICU admission independent of age.

CONCLUSION

A NLR > 3.6 is a useful variable to be included in risk prediction scores. In this study, we have highlighted the importance of NLR in COVID-19 patients in predicting disease severity and mortality. In a developing country like India, where there are limited resources, NLR can be used as an effective and economically cheap marker to predict and stratify COVID-19 patients as per severity and effectively predict the outcome as well, which in turn would lead to efficient resource utilization.

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