The role of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and neutrophil-to-platelet ratio as predictive markers of severity in COVID-19 patients

Running title: NLR, PLR, and NPR in COVID-19

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Abstract

Background: COVID-19 is a global pandemic caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). Various clinical and hematological findings that can predict disease severity have been identified. This study aims to explore the role of Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), and Neutrophil-to-Platelet Ratio (NPR) in predicting the severity of COVID-19 infection.

Methods: In this analytical cross-sectional single-center study, after obtaining Ethics Committee clearance, patients with laboratory-confirmed COVID-19 infection admitted during their first two weeks of illness were included in this prospective study. NLR, PLR, and NPR were derived from the CBC reports. These ratios were compared in each clinical category group to assess the severity.

Results: The total number of cases was 160, with a mean age at diagnosis of 56 years. The proportion of males was slightly higher (54.4%) than that of females (45.6%). The proportion of Category C patients (66.9%) was more than Category B (25%) and Category A (8.1%) patients. It was found that NLR, PLR, and NPR ratios had a statistically significant association with severe COVID-19 infection, suggesting they can be used to differentiate between Category C and Category A or B. NLR is a better parameter in predicting the severity of COVID-19 disease than PLR and NPR.

Conclusions: NLR, PLR and NPR ratios can be used as predictive markers of disease severity in COVID-19 infection. Among these ratios, NLR has the highest predictive value for disease deterioration.

Key words: Peripheral Smear, SARS-Cov-2, Covid-19, Neutrophil to Lymphocyte ratio, Platelet to lymphocyte ratio, Neutrophil to Platelet ratio.

Introduction

The SARS-CoV-2 virus, a highly contagious enveloped single-stranded RNA virus that belongs to the family of Beta coronaviruses is the causative agent of the infectious disease COVID-19. Most virus-infected individuals will experience mild to severe respiratory disease but will recover without the need for special care. However, some people will get fatal illnesses (1). From asymptomatic carriers to moderate respiratory symptoms and fatal acute respiratory distress syndrome, SARS-CoV-2 infections can affect anyone. Major sickness is more likely to affect elderly persons and people with serious co-morbidities, such as cancer, diabetes, cardiovascular disease, or persistent respiratory issues. Fever, cough, anorexia, dyspnea, sputum production, and myalgia are some of the most prevalent symptoms of the illness, according to the CDC (Centers for Disease Control). Uncertainty surrounds the true SARS- CoV-2 infection fatality rate, which has been reported to range from 0.3% to 8.4% globally. COVID-19 has the capacity to render someone seriously ill or result in their death at any age (2).

Several biomarkers have been identified in COVID-19. Many studies have reported on the predictors of disease severity in COVID-19 patients in light of the rise in COVID-19 cases across the globe as a result of its highly contagious nature. It has been shown that when compared to milder non-fatal cases, severe or fatal cases of COVID-19 disease are associated with elevated white cell count, lower lymphocyte count (1000/L), and lower platelet count (100x109/L) (3-6).

Anemia, polycythemia, leukopenia, leukocytosis with neutrophil predominance, and leukocytosis with decreasing platelet count have all been linked to severe illness and a worse prognosis in hospitalized patients. In the immunocompromised phase, the virus is found to cause T-cell immunological dysregulation, which results in monocyte/macrophage activation, uncontrolled cytokine release, and catastrophic multi-organ failure (7-10). There are close connections between the hematological, coagulation, inflammatory, and immune pathways. Different biomarkers are produced based on the organ or system of origin. The most important biomarkers that are elevated in COVID-19 are CRP, LDH, D-dimer, and Ferritin (11). This study aims to explore the role of Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to- Lymphocyte Ratio (PLR) and Neutrophil-to-Platelet Ratio (NPR) in predicting the severity of COVID-19 infection.

Methods

This analytical cross-sectional single-center study was conducted after obtaining the approval from Institutional Ethics Committee, at MES MEDICAL COLLEGE, in Perinthalmanna, Kerala. A total of 160 cases diagnosed as COVID-19 positive by Antigen test or RT PCR test and admitted during the period from August 1, 2020, to January 31, 2022, at MES Covid Hospital during their first two weeks of illness were included in this study. Antigen/ RT PCR test positive patients were admitted after 2 weeks of starting the symptoms, and patients with pre-existing hematological malignancies were excluded.

The enrolled patients were assessed upon admission and classified into three categories according to the World Health Organization (WHO) criteria. Category A represents mild disease, and includes patients exhibiting symptoms such as fever, malaise, cough, and upper respiratory issues, along with less common manifestations of COVID-19 like headache and loss of taste or smell.

Category B denotes moderate disease, characterized by the presence of lower respiratory symptoms, which may be accompanied by infiltrates visible on chest X-rays. Patients in this category are capable of maintaining adequate oxygenation while breathing room air.

Category C encompasses severe disease, where patients experience significant complications. Key indicators of severe illness include hypoxia, defined as an oxygen saturation (SPO2) of 93% or

lower on atmospheric air or a PaO2/FiO2 ratio below 300 mmHg. Additionally, tachypnea is present, indicated by a respiratory rate exceeding 30 breaths per minute, and more than 50% lung involvement is observed on chest imaging.

After obtaining informed consent, the demographic details were collected from study group. The clinical features including vitals and comorbidities, were recorded. The chest X-ray findings and the clinical diagnosis were noted.

Blood samples were collected from patients by venipuncture. Complete blood count (CBC) was done in Mindray BC-6200, automated 5 – part differential hematology analyzer. The ratios of neutrophil/lymphocyte count, platelet/lymphocyte count and neutrophil/platelet count were derived from the CBC report and evaluated.

NLR was calculated as the ratio of the absolute neutrophil count to the absolute lymphocyte count, PLR as the ratio of the platelet count to the absolute lymphocyte count and NPR as the ratio of absolute Neutrophil count to the absolute platelet count. The optimal cut-off values of the continuous NLR, PLR, and NPR were calculated by applying the receiver operating curve (ROC) analysis. The area under the curve (AUC), sensitivity and specificity of hematological parameters in predicting the severity were analyzed.

Statistical analysis

The data was entered in Microsoft Excel 2019. Proportions of various hematological parameters and the cellular characteristics were analyzed and compared in each clinical category group. Descriptive statistics included frequency analysis (percentages) for categorical variables and mean ± SD for continuous variables. Comparisons were done using the Chi-square test for categorical variables and the ANOVA test for continuous variables. Univariate logistic regression was performed to explore the association of clinical characteristics and laboratory parameters.

The ratios of neutrophil/lymphocyte count, platelet/ lymphocyte count, and neutrophil/platelet count were calculated. The optimal cut-off values of the continuous NLR, PLR, and NPR were determined by applying the ROC analysis. The AUC, sensitivity, and specificity of hematological parameters in predicting the severity were analyzed. Statistical analysis was performed using SPSS version 26. All the tests with a p-value
<0.05 was considered statistically significant.

Results

A total of 160 confirmed cases of COVID-19 were included in this study. These patients were categorized into severe (Category C) and non-severe (Category A and B) based on their initial clinical presentation at the time of admission. The non-severe group had 53 cases (Category A- 13 cases and Category B- 40 cases), while the severe group had 107 cases (Table 1).

The age distribution of the current study was found to be 56.91+ 15.75 with a minimum of 20 and a maximum of 88 years. The proportion of males (54.4%) was slightly higher than that of females (45.6%). Table 2 shows the distribution of vital signs among the study population. The mean systolic and diastolic BP were found to be higher than normal reference range.

High frequencies of severe cases were observed in patients with comorbidities like diabetes or hypertension. In this study, 35.63% (n=57) had no co-morbidities. The common co-morbidities observed in this study population were Type2 diabetes mellitus, hypertension, coronary artery disease and bronchial asthma (Table 3).

The present study analyzed the mean differences of various hematological parameters among participants with different categories of COVID-19. The mean total count and mean neutrophil count were found to be significantly higher among category C patients. The mean lymphocyte

count was found to be lowest among participants with category C, and this was also statistically significant. Platelet count showed no statistically significant difference among the three categories of COVID-19 (Table 4).

On analyzing the ROC curve, the area under the curve for NLR, PLR, and NPR was found to be above the reference line, which implies NLR, PLR, or NPR can statistically differentiate between the severe category of Covid-19 (Category C) from the non-severe ones (Category A or B) (Figure 1).

Since the area under the curve for the NLR ratio is higher than that of the PLR and NPR ratios, it can be considered as a better test to differentiate between Category C Covid-19 from Categories A and B. The cut-off value observed from the ROC curve for NLR was 3.680 with a Sensitivity of 70.1% and Specificity of 60.4%. The cut-off value for PLR was with a Sensitivity of 70.1% and a Specificity of 49.1%. NPR cut off was 0.0250 with a Sensitivity of 60.7%, and Specificity of 64.2% (Table 5).

Discussion

This study aimed to determine the role of NLR, PLR, and NPR ratios in predicting the disease severity and outcomes among the COVID-19 patients. The study population included patients diagnosed as COVID-19 positive by Antigen test or RTPCR test admitted during the period of August 2020 to January 2022 at MES Medical College, Perinthalmanna during their first two weeks of illness. A total of 160 participants satisfying the inclusion and exclusion criteria were included in the study.

The mean age of study population was 56.91+ 15.75 years with a range of 20 to 88 years. In this study, the proportion of males (54.4%) was slightly more than that of females (45.6%). A similar age distribution was observed in a study by Nazarullah A et al where the mean age was 55 years ranging from a minimum of 25 years and a maximum of 100 years. Among the COVID-19 positive patients in the study by Nazarullah A et al 58.33% were males and the rest were females. This finding is similar to the present study (12).

The mean differences of different hematological parameters among all three COVID categories were analyzed in the current study. According to the study, category C patients had significantly higher mean total counts and mean neutrophil counts. Category C had the lowest mean lymphocyte count, which was also statistically significant. There was no statistically significant difference among the three categories of COVID patients for platelet count. Another study by Dubey DB et al reported that patients with severe illness had significantly higher total leucocyte counts (TLC) than patients who only had mild or moderate symptoms. They found a significant difference in the mean Total leucocyte count, Neutrophil%, Lymphocyte%, and Monocyte% between cases with mild and moderate symptoms (13).

This study assessed whether the NLR, PLR, and NPR ratios can predict the disease severity in COVID. In the ROC curve, the area under the curve for NLR, PLR, and NPR was found to be statistically significant, which implies that NLR, PLR, and NPR can differentiate between category C patients from Category A and B. The area under the curve for the NLR ratio is higher than that of PLR and the NPR ratio. Hence, NLR can be considered as a better parameter to differentiate between category C COVID patients from Category A and B. For this, the cut-off values observed for NLR, PLR, and NPR ratios were 3.680 (sensitivity 70.1%, Specificity 60.4%), 146.50 (sensitivity 70.1%, Specificity 49.1%), and 0.0250 (sensitivity 60.7%, Specificity 64.2%), respectively. Yang A-P et al. observed a higher area of 0.841 under the ROC curve for NLR. For PLR, the area under the curve was 0.784. The highest specificity and sensitivity were 0.636 and 0.88 for NLR at a cut off of 3.3 and 0.44 and 0.77 for PLR at a cut-off of 180 in their study (14).

Another study by Asaduzzaman MD et al observed that in hospitalized COVID-19 patients, neutrophil-to-lymphocyte ratios (NLR), derived NLRs (d-NLR), and neutrophil-to-platelet ratios (NPR) were found to be significant predictors of mortality. In order to predict in- hospital mortality for Covid-19 patients, the optimal cut-off points for NLR, d- NLR, and NPR were 7.57,5.52, and 3.87, respectively, in their study (15).

In another study on 3508 COVID-19 patients, Chan A S et al observed that NLR and PLR ratios were statistically high in severe categories of COVID-19, as found in this study. The NLR value showed a Standard mean difference of 2.80, 95% Confidence interval of 2.12- 3.48 and p value of less than 0.00001 when compared with patients of non-severe category. Similarly, PLR also showed statistically significant association in predicting the severity of COVID-19 with a Standard mean difference of 1.82, 95% Confidence interval of 1.03- 2.61 and p value of less than 0.00001 (16).

The study on "the effects of NLR, NMR, NPR and CRP values on the patient's transmission to the intensive care unit and mortality" conducted by Akan O Y et al in a total of 160 COVID-19 positive cases, showed that NLR and NPR ratios have statistically significant association in COVID-19 as in our study. The cut off value of NLR in patients admitting to intensive care unit was 2.9 while the mortality cut-off value of NLR was 3.7. The cut-off values for NMR, NPR and CRP for mortality rate were 9.5, 0.022 and 79.2, respectively (17).

Conclusion

In this study we found that the NLR, PLR, and NPR ratios have statistically significant association with severe Covid-19 infection and hence these can be used to differentiate between Category C from Category A and B. NLR ratio has more area under the ROC curve and so can be considered as a better parameter to differentiate between Category C from Category A and B of Covid-19 infection.

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Ethics approval

Institutional Ethics Committee of MES Medical College, Perinthalmanna (Reference No. IEC/MES/10/2020, Dated 22/12/2020)

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

Author contribution

All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Distribution of COVID-19 categories among the study population. The majority (66.9%) of the study population were in COVID-19 category C, followed by category B (25%)

Category	Frequency	Percentage (%)
A	13	8.1
B	40	25
C	107	66.9
Total	160	100

Table 2. Distribution of vital signs among the study population

Parameter (Unit)	Minimum	Maximum	Mean	Standard deviation
SBP (mmHg)	110	200	141.89	16.353
DBP (mmHg)	70	144	93.22	15.129
PR (beats/min)	56	122	74.15	7.465
RR (per min)	16	28	21.65	2.419
SpO_2 (%)	80	100	91.44	4.239

Table 3. Comparison of comorbidity with COVID-19 severity categories

Comorbidity		COVID category		Total	P-value
	A	**B**	**C**		
Yes	7	17	79	103	0.001
No	6	23	28	57	
Total	13	40	107	160	

Table 4. Comparison of various parameters as quantitative variables

Parameter	Category	Number	Mean	Standard deviation	P-value
Total count	A	13	6.839231	2.1800553	0.027
	B	40	7.277750	3.2426584	
	C	107	9.318318	5.3708543	
	Total	160	8.606750	4.8175744	
Neutrophil count	A	13	5.176923	2.3931165	0.005
	B	40	5.194500	2.9694159	
	C	107	7.822430	5.4454838	
	Total	160	6.950500	4.8896213	
Lymphocyte count	A	13	1.326154	0.6388993	0.001
	B	40	1.688000	0.8247772	
	C	107	1.137196	0.6646120	
	Total	160	1.290250	0.7401385	
Platelet count	A	13	260.92	72.131	0.559
	B	40	221.55	84.737	
	C	107	241.08	143.098	
	Total	160	237.81	126.185	

Table 5. Prediction of severity category of COVID-19 using NLR, PLR, and NPR ratios

Test result variable	Cut off value	Area under the curve	Sensitivity (%)	Specificity (%)	P-value
NLR	3.680	0.702	70.1	60.4	< 0.001
PLR	146.50	0.658	70.1	49.1	0.001
NPR	0.0250	0.641	60.7	64.2	0.004

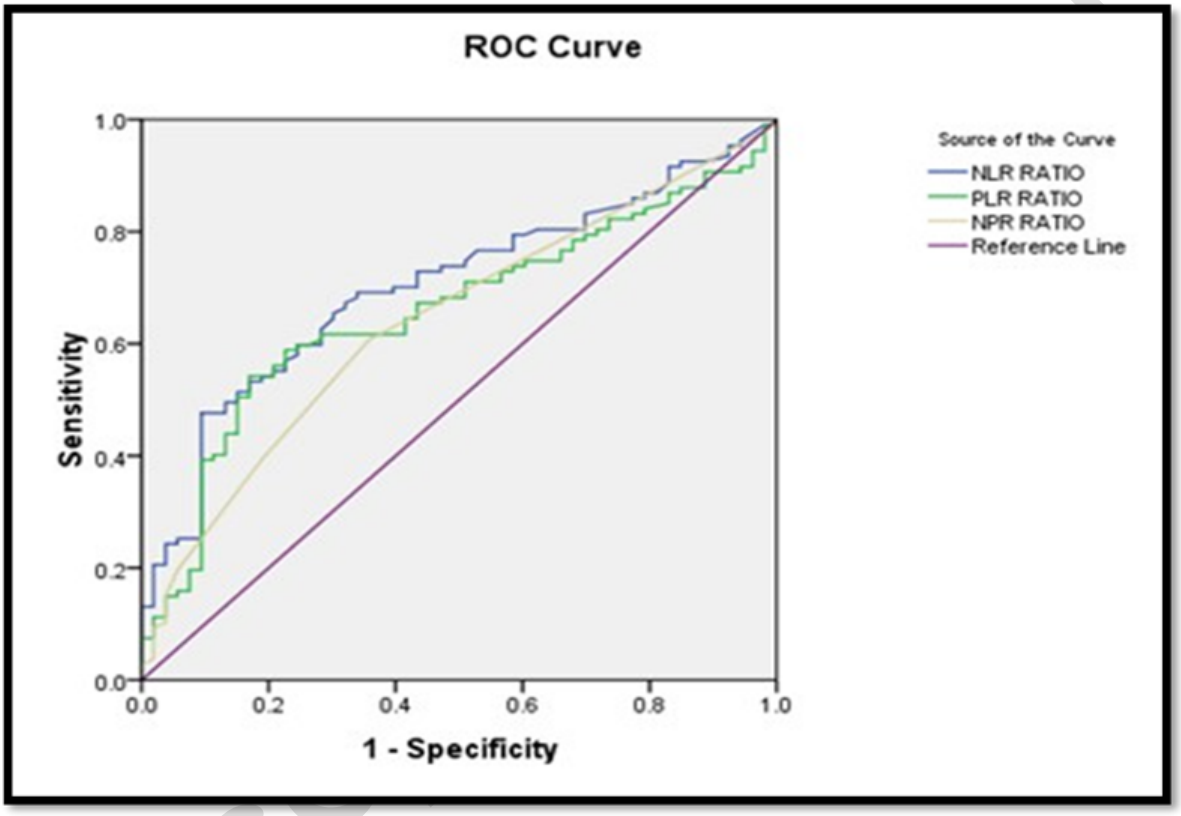

Figure 1. ROC Curve depicting NLR, PLR, and NPR ratio