



Utility of Neutrophil Lymphocyte Ratio (NLR) and Platelet Lymphocyte Ratio (PLR) as A Predictor of Mortality in COVID-19

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DOI: 10.31964/mltj.v0i0.434

Abstract: Coronavirus-19 pandemic has stricken our world since December 2019; the disease, first reported in China, is now a pandemic. More than 400 million people have been affected, and 5 million people have succumbed to the disease. Hence, it is the need of the era to find readily available laboratory parameters to assess the mortality chances in these patients. Our study aims to determine the utility of NLR and PLR ratios as a predictor of severity and clinical outcome of COVID-19 patients. 100 patients admitted to a tertiary care hospital in Karnataka, India, during the months April to July 2020 were studied. Only patients with a positive RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) report for COVID-19 were included. Demographic data, comorbidities, and mortality status were collected from electronic hospital records. Lab parameters including- Total Count (TC), Absolute count of neutrophils and lymphocytes, platelet count were taken. NLR and PLR were derived from available lab parameters. Patients were categorized into varying severity depending on their SpO₂ levels at admission. Neutrophil count (P=0.001) and NLR (P=0.002) were associated with an increased risk of mortality and disease severity. An increase in PLR ratio (P=0.05) shows a mild association with mortality but not with disease severity (P=0.096). In contrast, comorbidities, increasing age, and gender did not show any statistical significance for mortality. The presence of statistical significance concerning NLR and PLR should be utilized as an aid by clinicians to assess disease severity and chances of mortality. As new variants of the disease are uprising and a single therapeutic measure is not available currently for the treatment of COVID-19, clinicians should be well informed about how to monitor the disease in a cost-effective and easily accessible way to reduce the disease mortality and morbidity.

Keywords: COVID-19; Neutrophil Lymphocyte Ratio (NLR); Platelet Lymphocyte Ratio (PLR)

INTRODUCTION

Coronavirus disease (COVID-19) is an infection caused by the severe acute respiratory syndrome coronavirus 2. COVID-19 was first reported in China and soon spread to other countries and became a significant public health problem. For this reason, it was declared a pandemic by the World Health Organization (WHO).

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Coronavirus is known to cause severe respiratory infections in humans. COVID-19 is transmitted from person to person through direct contact or droplets (Erdogan et al., 2020). Despite significant improvements in medicine, we are still in our infancy regarding its management. COVID-19 infection can be categorized as asymptomatic, mild, moderate, and severe disease. Moderate and severe diseases have been associated with prolonged hospital stays, complex clinical management, and high mortality rates (Imran et al., 2020). Immunological studies show that high levels of proinflammatory cytokines, known as a cytokine storm, are the characteristic feature of severe COVID-19 cases. This extreme elevation of cytokines causes a massive proinflammatory response resulting in Multiple Organ Dysfunction Syndrome (MODS), leading to mortality. Therefore, these inflammatory markers can be used to assess the severity and mortality risk of COVID-19.

PLR is a new marker of inflammation, which is inexpensive and available in clinical settings. It has been used in various diseases, such as cardiovascular and autoimmune diseases, to predict inflammation and mortality. Due to the rapid involvement of inflammatory processes in COVID-19, severe patients have elevated PLR levels on admission (Simadibrata et al., 2020). Neutrophils constitute the majority of leukocytes and are responsible for activating the immune system. Viral antigens are exposed, and an antibody-dependent cell-mediated mechanism stimulates cell-specific and humoral immunities. There is increasing interaction with molecules like vascular endothelial growth factor (VEGF), interleukin-6, interleukin-8, tumor necrosis factor-alpha (TNF- α), interferon-gamma, and granulocyte colony-stimulating factor. VEGF-A and VEGF-C are particularly rigorous in the novel coronavirus. The immune response is massively dependent on lymphocytes for exerting a response.

On the other hand, systemic inflammation destroys CD4+ T lymphocytes and increases suppressor CD8+ T lymphocytes, leading to an increased neutrophil-to-lymphocyte ratio (NLR) (Bg et al., 2021). Most patients infected with the novel coronavirus had a mild and moderate illness, and severe illness was seen only after one week. In cases of critical illness, patients progressed rapidly into acute respiratory failure, acute respiratory distress syndrome, metabolic acidosis, and septic shock. Early identification of risk factors for critical illness is aided by the appropriate provision of care and rapid access to the intensive care unit (ICU) when required. General isolation treatment is required for patients with mild and moderate disease, and ICU care is not needed unless the condition worsens. Thus, early prognosis prediction would help reduce mortality and alleviate the shortage of medical resources (Liu et al., 2020).

Bearing all this in mind, our study aims to assess the utility of NLR and PLR (a readily available and inexpensive lab parameter) in determining mortality and severity in COVID-19 patients concerning their demographic and comorbidities. There have been studies only considering a single group of lab parameters in COVID-19. Our study has taken two ratios, NLR, PLR, and absolute counts, into consideration concerning mortality and severity of the disease. It is the first of its kind in our demographic population of central Karnataka, India, compared with other data from the world. Our study aimed to understand the utility of NLR and PLR ratios in assessing the mortality and severity among COVID-19 patients.

MATERIALS AND METHODS

This study is a retrospective cross-sectional study conducted in the Department of Pathology in a tertiary care hospital in Karnataka. We commenced the study after approval from Institutional Ethical Committee-JJM Medical College, Davangere,

Karnataka (IEC Registration No-ECR/731/Inst/KA/2015/RR-18 issued under Rule 122DD of the Drugs & Cosmetics Rules 1945. Ref No.:- JJMMC/IEC-06-2022) Clinical data and laboratory data(including- neutrophil count, platelet count, lymphocyte count, and the ratios like NLR and PLR derived from them) were recruited from electronic medical records. Data for four months- April to July 2020 was included. Patients of both genders above 18 years with positive RT-PCR reports and adequate clinical data were included. Patients were categorized into mild, moderate, and severe according to their clinical features and SpO2 at admission. A complete Blood Count was performed on Mindray BC-200.

These categories were: The mild disease was defined as symptoms of fever, sore throat, cough, and SpO2 > 90%. The moderate disease was defined as fever and respiratory symptoms and SpO2 > 80%. Severe diseases are patients with respiratory distress (respiratory rate >30 breaths per min, SpO2< 80%.

Our study selected 100 in-patient COVID-19 cases who were RT-PCR positive. Categorical data were represented in the form of frequency and percentage. The Association of variables was assessed with the Chi-Square test. Quantitative data were defined as mean and standard deviation. The comparison was made with an unpaired t-Test and Kruskal Wallis Test. A P-value of <0.05 was considered statistically significant, P-value of <0.001 was considered highly significant. IBM SPSS Version 22 for Windows was used for analyzing the data.

RESULTS AND DISCUSSION

Out of 100 patients considered, 56 were males, and 41 were females. 76.2%,19.1%, and 6.7% of the severe, moderate, and mild category patients, respectively, succumbed to the disease. Thereby establishing that increased mortality is associated with higher disease severity(P<0.01) in table 1.

Table.1 Association between Severity of Disease and Mortality

Severity	Outcome		Chi Square Test
	Died	Survived	P Value
Mild	6.7%	93.3%	P<0.001
Moderate	19.1%	80.9%	
Severe	76.2%	23.8%	

Patients were seen at a maximum of 40-59 (47%). Although the percentage of mortality was found to be higher in the older age group that is, 37.5%, 26.6%,19.9%, and 26.6% in the age groups > 80years, 60years-79 years, 40-59 years, and 20-39 years, respectively, it was not statistically significant (P=0.664) concerning mortality in table 2.

Table. 2 Moratlity among Different Age Groups

Age (In Yrs)	Outcome	
	Died	Survived
20-39	26.6%	73.4%
40-59	19.9%	80.1%
60-79	26.6%	73.4%
≥ 80	37.5%	62.5%

The presence or absence of any comorbidities, including non-communicable diseases (P=0.292), didn't show any statistical significance concerning mortality. NLR and PLR being novel parameters, have no research data available regarding their normal range in the research demographic population till now. Studies from China have found normal ranges for the same; that is, NLR in normal males and females is 0.43~2.75 and 0.37~2.87, PLR is 36.63~149.13, and 43.36~172.68, respectively. (Wu et al., 2019)

Laboratory parameters like neutrophil count (NC) was higher in patients who died of the disease (mean:14052.08 among died; mean: 6844.32 among survived), and NLR was much higher in the patients who succumbed to the disease (mean:19.5 among passed; mean: 9.78 among survived) showed high statistical significance concerning mortality (P<0.001). Whereas PLR (mean:329.41 among died; mean:254.33 among survived) showed statistical significance (P<0.05). Parameters like lymphocyte count (LC) (P=0.240) and platelet count (PC) (P=0.248) do not show any statistical significance table 3.

Table 3. Laboratory Parameters Compared with Mortality

Parameters	Died		Survived		Unpaired t Test
	Mean	Std. Deviation	Mean	Std. Deviation	P value
NC	14052.08	6910.09	6844.32	4481.90	<0.001
LC	910.88	566.30	1064.38	550.15	0.240
PLC	2.43	0.93	2.16	0.99	0.248
NLR	19.59	13.95	9.78	12.64	<0.001
PLR	329.41	171.56	254.33	166.99	<0.05

Parameters like NC shows an increasing trend with higher disease severity (Mean of NC among mild, moderate, and severe being-6970.66 ± 5054, 8059.1 ± 3766.9, and 13518.1 ± 7565.0 respectively) with a P-value of <0.001 and NLR, even though is high in all three severity groups increases with higher disease severity (Mean NLR among mild, moderate and severe being- 9.99± 14.1, 10.3 ± 7.42 and 19.9 ± 14.4) with a P-value of <0.001-thereby showing high statistical significance concerning the severity of disease; whereas parameters like LC, PLC and PLR (with P=0.07, 0.968 and 0.096, respectively) didn't show any statistical significance table 4.

Table 4. Laboratory Parameters Compared with Disease Severity

Parameters	Severity			Kruskal-Wallis H Test
	Mild	Moderate	Severe	P Value
NC	6970.66 ± 5054	8059.1 ± 3766.9	13518.1 ± 7565.0	P<0.001
LC	1112.8 ± 605.4	995.7 ± 418.9	823.9 ± 489.9	0.070
PLC	2.26 ± 1.1	2.13 ± 0.71	2.23 ± 0.89	0.968
NLR	9.99 ± 14.1	10.3 ± 7.42	19.9 ± 14.4	P<0.001
PLR	257.2 ± 168.1	257.1 ± 160.6	329.4 ± 181.3	0.096

Our study shows that high neutrophil count and NLR, which might be a result of excessive inflammation and immune suppression in sepsis triggered by SARS-CoV-2 infection, can be useful for predicting its severity and mortality. The potential reason for this phenomenon may come from the physiological response of innate immunity to systemic inflammation. It has been reported that ACE2 (Angiotensin-converting enzyme 2) is the receptor of SARS-CoV-2 and plays a crucial role in the infection, lymphocytes that express the ACE2 may be a direct target of viruses and are vulnerable to being attacked, and SARS-CoV-2-induced NKG2A expression may be correlated with functional exhaustion of cytotoxic lymphocytes at the early stage, which may result in disease progression (Zhao et al.,2021).

In sepsis, neutrophils are hyperactivated with delayed apoptosis, along with the depletion and exhaustion of CD4 and CD8 T cells resulting from apoptosis are everyday events in severe COVID-19. Partly due to T-cell apoptosis, lymphocytopenia is also common in SARS-CoV infection. Compared with other tests, NLR is an efficient and practical indicator of the risk of COVID-19 mortality (Liao et al.,2020). Many studies agree that increased NLR is an independent prognostic factor for COVID-19 patients, especially those older than 50. Studies have also shown NLR to help predict outcomes of different types of infections, including community-acquired pneumonia, bacteremia, and endocarditis. NLR has been studied and proved helpful in differentiating between patients hospitalized with fever due to illness and those with fever due to non-infectious causes (Prozan et al., 2021).

Our study also shows PLR to have significance concerning mortality. A higher PLR is mainly caused by megakaryocytes in the hematopoietic tissue and is a major participant in thrombosis. It plays an essential role in the inflammatory response to recruit neutrophils and other inflammatory cells to the injury site. Platelets exist in an inactive form and can be activated quickly at the site of vascular injury and can be rapidly activated in response to proinflammatory cytokine or infectious factors. Lymphocytes are among the primary immune-active cells in the human body, and lymphocyte count is an early marker of stress and systemic inflammation.

The advantage of PLR selection is that it reflects both aggregation and inflammatory pathways and maybe more valuable in predicting various inflammations than platelet or lymphocyte counts alone (Qu et al.,2020). In a study performed in China (Yanga et al.,2020) which considered 69 non-severe and 24 severe cases of COVID-19, NLR and age were found to be significantly associated with illness severity. The binary logistic analysis identified increased NLR (hazard risk [HR] 2.46, 95% confidence interval [CI] 1.98–4.57) and age (HR 2.52, 95% CI 1.65–4.83) as independent factors for poor clinical outcome of COVID-19, which is in agreement to our study where NLR is statistically significant concerning disease severity. Still, age is not associated with increased disease severity.

In a retrospective study conducted in Pakistan (Taj et al., 2020) showed that out of 101 patients, 20.8%, 51.8%,19.8%, and 7.9% were in mild, moderate, severe, and critical groups. Median (IQR) values of WBCs (P=0.004), ANC (P=0.002) and NLR (P= 0.001), were increased in critically ill patients. A study was done in Iran among 219 patients (Moradi et al., 2020) showed increasing age and presence of comorbidities is associated with a higher mortality rate, which is in disagreement with our study- probably due to a lower number of patients in higher age group and decreased presence of the comorbidities in the admitted patients in our hospital setting. In a study (Escobar et al., 2021) comprising 2088 patients, parameters like PLR, NLR, NPR (Neutrophil Platelet Ratio) were significantly increased in severe cases of COVID-19 disease.

In another study, both NLR and PLR ($P < 0.01$) correlate with disease severity positively. In contrast, in our study, NLR was statistically significant concerning the severity of infection, while PLR ($P = 0.096$) didn't show any statistical significance concerning severity. In an Indonesian study (Suastika et al., 2021), 507 COVID patients were considered PLR to be significantly higher in severe cases compared to mild-moderate cases ($p < 0.001$). The cut-off value of PLR to predict severe cases of Covid-19 is more than 150, with a sensitivity of 80.5%, a specificity of 66.3%, and an area under the curve (AUC) of 0.807 ($p < 0.001$, 95% confidence interval (CI) 0.759 - 0.855). But our study shows that PLR has no association with significance concerning the severity of disease (P value=0.096).

The study's limitations include the absence of normal control groups and the limited sample size with all laboratory parameters. Further studies are to be done -to assess the sensitivity and specificity of these ratios.

CONCLUSION

This study found statistically significant tools, like NLR and PLR, to assess patient mortality. NLR was also found to aid patient disease severity, which was not appreciated with PLR and disease severity. As COVID-19 is highly infectious and different mutant strains appear over time, easily accessible parameters are the need of the day to monitor patients, especially those belonging to the moderate to severe category. By utilizing these parameters, clinicians should categorize patients depending on their severity to provide adequate and timely care.

CONFLICT OF INTEREST

The authors disclose no conflicts of interest.

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