

COVID-19 induced hematological derangements: An overview into Neutrophil-Lymphocyte Ratio and Platelet Indices and its relationship to age and sex of the patient.

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Abstract

Background: COVID-19 sets off a widespread inflammatory response in the body, which is reflected in various blood parameters. This study aims to explore the utility of neutrophil-to-lymphocyte ratio (NLR) and platelet indices such as mean platelet volume (MPV), and platelet lymphocyte ratio (PLR) as a prognostic tool by investigating its relationship to the age and sex of the patient.

Methodology: This laboratory-based retrospective study includes 510 RT-PCR diagnosed positive cases of COVID-19 during the first wave in Ramaiah Teaching Hospital, Bangalore which included patients above the age of 18 years of both genders. The NLR and platelet indices were derived from the values obtained from the automated hemoanalyzer. Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Chi-square test or Fischer’s exact test was used as test of significance for qualitative data.

Independent t-test was used as test of significance to identify the mean difference between two quantitative variables. P value of <0.05 was considered as statistically significant.

Results: Of the 510 cases, most patients (41.8%) fall within the age group 40-59 years with male : female ratio being 1.6:1. It is observed that mean NLR and PLR are significantly higher in the COVID-19 affected cases included in the study while MPV was slightly below the normal range. Males are found to have higher NLR and PLR (p<0.004 and p<0.016 respectively). All three parameters are found to be statistically significant in patients above the age of 60 years.

Conclusion: NLR and PLR increases and MPV decreases in the setting of COVID 19 infection, and its deviation from normal is more pronounced in males and older individuals. This throws light to its association to disease severity and hence, its use as a potential

biomarker of inflammation which is cost and time effective.

Keywords: COVID 19, Mean platelet volume, Neutrophil lymphocyte ratio, Platelet Lymphocyte ratio

Introduction

Corona virus disease 2019 (COVID-19), an infection caused by a novel corona virus known as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) which was initially detected in Wuhan City, Hubei Province, China.(1) As reported to WHO, India has seen 43,029,044 confirmed cases of COVID-19 with 521,358 deaths, from January 2020 to April 2022.(2) COVID-19 sets off a widespread inflammatory response in the body, the aftermath of which depends on several host factors. Therefore, circulating biomarkers that can represent inflammation and immune status are potential predictors for the prognosis of COVID-19 patients. (3)

The purpose of this study is to analyse the hematological derangements occurring in COVID with respect to Neutrophil Lymphocyte Ratio (NLR) and Platelet Indices such as Mean Platelet Volume (MPV) and Platelet Lymphocyte Ratio (PLR) and how it varies with age and sex of the patient. In a developing country like India, these parameters can easily be employed as a time and cost - efficient prognostic tool in triage and resource allocation, especially considering the unpredictability of future outbreaks.

Aims And Objectives

1. To study the variations of NLR, MPV and PLR of COVID-19 patients.
2. To investigate the relationship of these parameters to the age and sex of the patient.

Materials And Methods

This laboratory-based retrospective study includes 510 RT-PCR diagnosed positive cases of COVID-19 during the first wave in Bangalore, Karnataka from March to June 2022. Patients above the age of 18 years of both genders were included in the study. Relevant patient demographics were collected from the medical records. The NLR and platelet indices are derived from the values obtained from the automated hemoanalyzer of the pathology laboratory of Ramaiah Teaching Hospital, Bangalore. Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software (IBM SPSS Statistics, Somers NY, USA). Categorical data was represented in the form of Frequencies and proportions. **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. **Independent t test** was used as test of significance to identify the mean difference between two quantitative variables. **ANOVA** was used as test of significance to identify the mean difference between more than two quantitative variables. **P value** (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Results

Table 1 - Distribution of subjects according to age group

	Frequency	Percent
<18yrs	14	2.7
18-39yrs	160	31.4
40-59yrs	213	41.8
60yrs & above	123	24.1
Total	510	100.0

Table 2 - Distribution of subjects according to sex

	Frequency	Percent
Female	194	38
Male	316	62
Total	510	100.0

Table 3 - Descriptive statistics of various parameters

	Minimum	Maximum	Mean	SD
Age (in yrs)	18	89	47.2	16.10
Total Count	1800	47800	8472.16	4813.43
Neutrophil (%)	5.4000	96.4000	65.04	16.273
Lymphocyte (%)	1.2000	88.2000	30.081	130.701
NLR	.0495	73.6667	5.744580	9.00
PLT	18000	766000	254105	105483
MPV	6.4000	30.3000	8.638824	1.380
PLR	9.47	1785.00	202.57	176.03

Table 4 - Comparison of various parameters according to Sex

	Female		Male		P value
	Mean	SD	Mean	SD	
Total Count	8534	4482	8433	5025	0.819
Neutrophil (%)	62.8172	15.2115	66.3639	16.7914	0.017
Lymphocyte (%)	26.6599	12.6705	32.2108	165.8132	0.644
NLR	4.2781	6.0261	6.6465	10.3306	0.004
PLT	276755	110715	240482	100349	0.001
MPV	8.6990	1.0297	8.6060	1.5583	0.463
PLR	178.7025	144.8763	217.4157	191.7155	0.016

Table 5:- Comparison of various parameters according to age group

	<18yrs		18-39yrs		40-59yrs		60&above		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Total Count	6579	2121	8446	5254	8199	4317	9195	5173	0.131
Neutrophil (%)	40.37	9.17	59.561	14.77	65.54	15.93	74.13	13.30	<0.001
Lymphocyte(%)	44.95	7.83	47.82	232.00	23.62	13.43	16.4911	10.3259	0.173
NLR	.9494	.35	4.0131	8.4748	5.64	8.412	8.7100	10.2939	<0.001
PLT	261929	62744	237706	93974	259733	110928	264659	112498	0.123
MPV	8.6500	.7969	8.8763	1.9926	8.5831	1.0086	8.4252	.9019	0.045
PLR	94.14	22.0	137.26	115.15	213.2	166.2	281.289	224.923	<0.001

A total of 510 cases were analysed. Most of the patients were within the age group 40-59 years (41.8%). Males constituted about 62% of the total cases with a male: female ratio of 1.6:1.

The mean NLR was observed to be 5.74 ± 9.00 . Males had a higher mean NLR of 6.64 ± 10.33 as opposed to women who's mean NLR was 4.27 ± 6.02 . This finding was statistically significant with a p value of 0.004. Mean NLR was recorded to be 8.71 ± 10.29 in the age group 60 years and above and is statistically significant. ($p < 0.001$)

MPV was found to be 8.60 and 8.69 in males and females respectively, a finding that is not statistically significant ($p = 0.46$). However, compared to all age groups, it was observed that in the age group 60 years and above, MPV was the least with a value of 8.42 ± 0.90 and is statistically significant. ($p = 0.045$)

Males had a mean PLR of 217.41 ± 191.71 and females 178.70 ± 144.87 and this difference is statistically significant ($p = 0.016$). PLR is also observed to increase with increasing age and is highest in patients of age 60 years and above (281.28 ± 224.9), a finding that is statistically significant with a p value < 0.001 .

Discussion

COVID 19 is clinically and radiographically similar to several viral diseases which compels the need to explore parameters other than RT PCR which could be characteristic to the disease. This study intends to explore the utility of NLR, MPV and PLR as useful circulatory biomarkers of COVID 19 associated inflammation and how it varies with age and sex of the individual.

The NLR is a simple and accessible inflammatory marker with a reference range of between 0.78 and 3.53. Our study reveals a mean NLR of 5.74 which is

evidently higher than normal. This is in concordance to a study by Prozan et al that suggested that NLR is even more a valuable prognostic marker in COVID-19 patients, than in other viral infections by influenza and RSV.(4) A high NLR implies an aberrant immune response, with increased neutrophils and decreased lymphocytes. (5) Neutrophil production can be triggered by virus-related inflammatory factors, such as interleukin-6 and interleukin-8, tumor necrosis factor- α , granulocyte colony stimulating factor, and interferon- γ . Lymphopenia is common in COVID-19, as a result of direct cytokine—induced inhibition.(6) The massive over—production of these cytokines, or "cytokine storm", can result in acute respiratory distress syndrome (ARDS), the hallmark of severe cases of COVID-19.(7) We also noticed higher NLR in patients of older age group, which is similar to a study by Rasyid et al. Aging causes major changes in immune response dysregulation, leading to a chronic systemic inflammatory state. Cytokines and chemokines also play a major role in the development of chronic inflammation and immunosenescence. However, there is a limited number of studies evaluating the effect of aging on NLR as part of the complete assessment for intensive care requirement and mortality risk. Age significantly affected endpoints in the low-risk groups. Whether age is an independent risk for or if comorbidities play a role with increasing age in COVID-19 mortality and severity is still debatable.(9)

Throughout this study, it was observed that males constituted more than half of the study population and had higher NLR compared to females. This higher incidence and severity in males have been studied by Ortolan et al, who suggested that this could be due to differences in innate immunity, steroid hormones, and

factors related to sex chromosomes. There is also a role of estrogen in the immune response to viruses. The presence of estrogen receptors on the surface of innate immune cells such as monocytes, macrophages, and neutrophils, facilitates the production of type I and III interferon by innate immune cells, which is crucial for decreasing virus titres. (10)

MPV is a measure of the average size of platelets, and the normal range is 9-12.4 fL. (11) The mean MPV was observed to be 8.63 ± 1.3 fL in the present study. Ozelik *et al.* reported statistically significantly ($p = 0.027$) decreased MPV in COVID-19 cases, which supports our finding. MPV levels are increased in several diseases but reduced in viral diseases due to destruction of young platelets at the site of inflammation as a result of increased inflammatory cytokine activity.(12) MPV was found to be the least in the age group above 60 years, presumably due to the severity of inflammation. A few studies conflict this finding where a high MPV is observed in COVID, as a response to thrombocytopenia.(13) This is probably due to the fact that in our study, many patients had nearly normal platelet count.

PLR is defined as the ratio of platelet to absolute lymphocyte counts. The normal range for PLR is 36.63 – 149.13%. In our study, the mean PLR was 202.57 ± 176.03 . A study by Simadibrata *et al.* found increased PLR in severe COVID-19 cases. PLR has been advocated as a novel biomarker for predicting the prognosis and severity of COVID-19. The underlying pathogenesis of high PLR levels in severe COVID-19 cases is not clearly understood. A possible explanation is a decline in absolute lymphocyte count, which may be greater than the decline in platelet count, leading to increased PLR in severe cases.(12) It was also noted that

PLR was significantly higher in males and older individuals. Löhr et al who discussed the positive influence of female gender on the level of CD4+ lymphocytes in human peripheral blood. Aging also has a negative effect on the number of CD3+ and CD8+ lymphocytes. This decrease in lymphocytes is more significant than the fall in platelets, which explains the higher PLR in men and older patients.

Conclusion

This study portrays the characteristic changes in NLR, MPV and PLR in COVID 19 patients and how it varies with the age and sex of the patient. The findings in this study supports other studies on the same by showcasing the association of these parameters to the severity of the disease. This throws light to their potential use as inflammatory biomarkers in COVID 19 to help triage and predict prognosis of the patient.

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