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Prognostic and predictive significance of neutrophils lymphocytes ratio (NLR), lymphocytes monocytes ratio (LMR), Platelets lymphocytes ratio (PLR) and C-reactive protein (CRP) in patients with confirmed COVID-19 infection

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Abstract
Background and objectives: Pneumonia cases of unknown origin were recorded and reported by several local health facilities in Wuhan, China, in December 2019. The etiological agent was identified to be the SARS-CoV-2; COVID-19. Laboratory tests reveal normal or increased total leukocyte count, reduced lymphocyte count has been seen in some severe cases. PLR between severe and non-severe cases has been studied and said to have prognostic significance. NLRis another significant indicator of detecting inflammation in routine laboratory tests among Covid-19 patients which can be utilized in the diagnosis, treatment and evaluation of prognosis. In addition to NLR, CRP levels have been found to be deranged in moderate and severe Covid-19 infections. **Material and Methods**: 82 laboratory confirmed Covid -19 cases, diagnosed by positive RT-PCR for SARS CoV-19 RNA on nasal/ oropharyngeal swabs were included in the study. **Results:** The optimal cut-off values for NLR and PLR were 4.63 and 0.167 respectively. For NLR curve, the results showed that AUC was 0.848 (95% CI, 0.766 - 0.931), the Youden index was the largest 0.543, corresponding to the best cut-off value of 4.63, the sensitivity was 0.787, and the specificity was 0. 755. Similarly, for PLR the area under the curve (AUS) was 0.772 (95% CI, 0.666 – 0.879), the Youden index was the largest 0.493, corresponding to the best cut-off value of 0.167, the sensitivity was 0.636, and the specificity was 0.857.**Conclusion:**The present study has re-emphasized the prognostic and predictive significance of routine hematological investigations and CRP in Covid-19 patients.
Keyword: neutrophil, CRP, Pneumonia.

Introduction

Pneumonia cases of unknown origin were recorded and reported by several local health facilities in Wuhan, China, in December 2019. The etiological agent was identified to be the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; COVID-19), later classified as an unknown beta-coronavirus[1].Patients commonly present with fever, nonproductive cough, fatigue and dyspnea. They have been found to have abnormal peripheral blood examination results. Peripheral blood counts especially eosinophils have been found to be useful in identifying and anticipating severe cases[2]. Laboratory tests reveal normal or reduced total leukocyte count, reduced lymphocyte count, thrombocytopenia along with elevated transaminase, lactate dehydrogenase (LDH), creatine kinase, and myoglobin levels. Reduced lymphocyte count has been seen in some severe cases. Comparison of platelet-to-lymphocyte ratio (PLR) between severe and nonsevere cases has been studied and said to have prognostic significance. It has also been thought to flag cases which should be

kept under surveillance and be therapeutic targets[3]. Neutrophil-Lymphocyte Ratio (NLR) is another significant indicator of detecting inflammation in routine laboratory tests among Covid 19 patients which can be utilized in the diagnosis, treatment and evaluation of prognosis[4]. In addition to NLR, serum amyloid A (SSA), C-reactive protein (CRP) and serum albumin (ALB) levels have been found to be deranged in moderate and severe Covid 19 infections[5]

Material and Methods

The study was commenced after approval by the Institutional Ethics Committee.   This study was aimed to evaluate the prognostic and predictive significance of NLR, LMR, PLR and CRP in patients with confirmed COVID-19 infection.It was a prospective analytic single centre observational study was conductedon 82 laboratory confirmed Covid -19 cases, diagnosed by positive RT-PCR for SARS CoV-19 RNA on nasal/ oropharyngeal swabs. Thirty three severe and fifty two non-severe Covid-19 cases admitted in ICU, HDU and isolation wards were included in the study. Both symptomatic and asymptomatic cases were included. They were divided into non-severe (asymptomatic or mild patients) and severe symptomatic patients based on Clinical Management Protocol: Covid-19 of Ministry of Health and Family Welfare (MOHFW), Government of India (GOI) (version5; 03 July 2020)Data was collected pertaining to epidemiological characteristics and medical records of patients including demographic data, underlying comorbidities, clinical signs

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and symptoms and laboratory tests including blood counts. Medical records of the patients were analysed in detail.WBC count, NLR, LMR and PLR were calculated from EDTA samples run on Medonic M20 GP.
CRP estimation was done by turbidity method on LABKIT (Spain) on SelectraProSElitech.Co-morbidities and old age being confounders, were marked as such and tackled appropriately during data analysis.Predictive value of the mentioned parameters was assessed by evaluating them with clinical severity at presentation as per laid down ICMR guidelines.Statistical analysis was done onSPSS 15.0 software (SPSS Inc, Chicago, USA).Chi-square and Fisher's tests were used for categorical variables while Wilcoxon rank sum tests were used for continuous variables. The optimal cut-off values of the continuous NLR, PLR, LMR and CRP were calculated by applying the receiver operating curve (ROC) analysis. P <0.05 was considered as statistically significant.

Results
A total of 82 patients were included in the study, out of which 49 patients were in the non-severe group and 33 patients in the severe group. The age range was 78 years to 18 years across the groups with 78 years being the oldest patient (severe group) and 18
years being the minimum age (non- severe group).The average age of the patients in the non-severe group was 36.04±12.84 years with 28

males and 21 females, whereas the average age of the patients in the severe group were 48.36±14.61 years with 24 male and 09 female patients. There were more males in the severe group. The differences in age between the two groups were found to be statistically significant (P < 0.05), but the difference in the gender was statistically non-significant (p value >0.05).
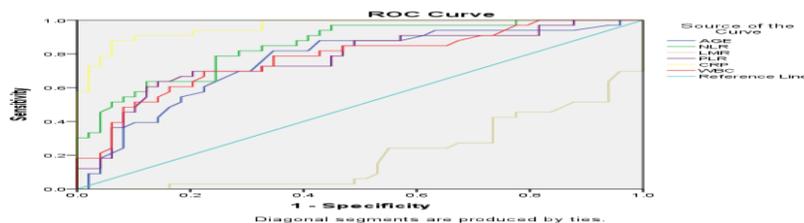
Among the non-severe group only 12% of the patients had associated co-morbidities (6/49), while in severe group 54% patients had associated comorbidity (18/33). It shows that patients with associated co-morbidity had more severe disease, and it was found to be statistically significant with p value <0.001. All the patients in the severe group were symptomatic at the time of presentation, while in the non-severe group, among 49 patients 36 patients was symptomatic and 13 were asymptomatic. WBC count, neutrophil count, lymphocyte count and CRP level were significantly high in severe group compared to the non-severe group (p value <0.05). (Table - 1)The most common presenting complaint among severe group was fever (63% of cases) followed by shortness of breath, which was seen in 54% of cases. While in non-severe case 46% of cases had fever at the time of presentation.

Table 1: Baseline characteristics and results of NLR, PLR, WBC, CRP patients

	Total (n=82)	Non-severe (n=49)	Severe (n=33)	P value
Age (Years) (M ± SD)	41±14.80	36.04±12.84	48.36±14.61	P value - <0.001
Sex (M/F)	52/30	28/21	24/09	P value - .151
WBC (Cells/mm3) (M ± SD)	9390.58±5272.36	7455.71±3057.82	12264.55±6487.49	P value - <0.001
Neutrophils (%)	72.51±13.30	66.06±12.16	82.09±8.21	P value - <0.001
Lymphocytes (%)	19.51±11.59	24.69±11.35	11.82±6.70	P value - <0.001
Monocytes (%)	3.04±1.49	2.98±1.50	3.12±1.49	P value – 0.67
NLR (M ± SD)	6.23±5.64	3.70±2.65	9.99±6.76	P value - <0.001
LMR (M ± SD)	8.39±7.62	11.03±8.56	4.47±3.23	P value - <0.001
PLR (M ± SD)	234.79±584.24	114.99±110.27	412.68±889.47	P value – 0.023
CRP (mg/L) (M ± SD)	51.65±80.29	9.64±11.91	114.03±96.90	P value - <0.001
Co morbidities n (%)	24(28)	06(12)	18(54)	P value - <0.001
Diabetes Mellitus	08(9.7)	01(2)	07(21.2)	-
Hypertension	12(14.63)	01(2)	11(33.3)	-
Hypothyroidism	06(7.3%)	04(8.1%)	02(6.06)	-
CAD	01((1.2)	00	01(3.03)	-
CKD	03	00	03(9.09)	-
Others	04(4.87)	01(2)	03(9.09)	-

We analysed the optimal cut-off values for NLR, PLR, LMR and CRP. It was calculated by ROC analysis, and the ROC curves are presented in Fig. 1. Areas under the curve (AUC) of age, WBC, NLR, CRP, LMR and PLR were 0.759, 0.775, 0.848, 0.960, 0.202, and 0.772. LMR could not be used as a potential diagnostic biomarker for subsequent analysis because its AUC was less than 0.50. The optimal cut-off values for NLR and PLR were 4.63 and 0.167 respectively.

NLR and PLR as prognostic and predictive marker in severe COVID-19 pneumonia and critical value judgment: As seen in the ROC curve to calculate the area under the NLR curve, the results showed that AUC was 0.848 (95% CI, 0.766 - 0.931), the Youden index was the largest 0.543, corresponding to the best cut-off value of 4.63, the sensitivity was 0.787, and the specificity was 0.755 (Figure 1). Similarly, for PLR the area under the curve (AUS) was 0.772 (95% CI, 0.666 – 0.879), the Youden index was the largest 0.493, corresponding to the best cut-off value of 0.167, the sensitivity was 0.636, and the specificity was 0.857 (Figure 1). The optimum cut off of Age, WBC count, and CRP were 37.5 years, 9650 cells/mm3 and 28.8 mg/L, respectively.

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Table 2: Area Under the Curve (AUC)
Area Under the Curve (AUC)

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
AGE	.759	.055	.000	.651	.867
NLR	.848	.042	.000	.766	.931
LMR	.202	.049	.000	.106	.298
PLR	.772	.054	.000	.666	.879
CRP	.960	.019	.000	.923	.997
WBC	.775	.053	.000	.670	.879

The test result variable(s): AGE, NLR, LMR, PLR, WBC have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Discussion
In this study, the age range was 78 years to 18 years across the groups, with 78 years being the oldest patient (severe group) and 18 years being the minimum age (non- severe group).The average age of the patients in the non-severe group was 36.04±12.84 years. Age distribution across the two categories was found to be statistically significant in our study with a P value of <0.001. We had 28 males and 21 females in the non severe group.On the other hand, the average age of the patients in the severe group were 48.36±14.61 years with 24 male and 09 female patients. Difference between genders across the two groups was statistically insignificant in our study.In the study by Shang et al on 443 Covid patients, there were 220 males and 223 females. The median age of the patients in the study was 56 years, with the interquartile range being 43.25-66.75 years. The severe category included 139 cases and the nonsevere group had 304 patients. They found statistically significant difference in the occurence across males and females, which was not affected by the confounding effect of heart disease with P < .05[6]. A study correlating PLR with prognosis in Covid 19 patients on 30 patients had the median patient age of 50.5 years, with the range extending from 36-65 years. They studied 16 males and 14 females with 27 patients in the non severe category and 3 in the severe category. They reported the age of patients as 49.44 ± 14.86 years in the non severe group and 60 ± 5.29 years in the severe group, with P value of 0.041[3]. Wang et al conducted a study on 45 COVID-19 patients, who were segregated into moderateand severe categories, including 35 (77.8%) and 10 cases (22.2%) respectively. They did not find any significant difference in the median age between the two groups (P>0.05). They had 23 male (51.1%) and 22 (48.9%) female patients in the study with insignificant statistical difference among the gender distribution across the two groups (P>0.05)[7]. Total leukocyte count was also found to be significantly different across non severe and severe categories with the average counts ranging from 5.03 x 10⁹ /L (4.05-6.33 x 10⁹ /L) and 6.01 x 10⁹ /L (4.20-7.80 x 10⁹ /L) respectively with a P value of 0.002 by Shang et al[6].Average Total Leukocyte Count across the groups averaged at 9390.58±5272.36 cells/mm³ and was 7455.71±3057.82 cells/mm³and 12264.55 ±6487.49 cells/mm³ in the non severe and severe categories respectively with a P value <0.001. WBC counts were found to be significantly higher in the severe patients (P<0.05)in the study by Wang et al[7]Feng et al, in their systematic review and meta analysis on Immune-inflammatory parameters in COVID-19 found that in comparison to patients with normal total leukocyte count, those who had elevated TLC had three times greater chances of having severe disease[8]. We found percent neutrophil counts to be 72.51±13.30 across the groups with the values being 66.06±12.16 % in the non severe group and 82.09±8.21% in the severe group with a statistically significant P of<0.001.Likewise, average lymphocyte count(%) overall was found to be 19.51±11.59 by us and varied from 24.69±11.35 to 11.82±6.70 across the non severe and severe categories respectively (P<0.001). Monocytes (%) were found to be 3.04±1.49 on an average across the two groups, but varied from

2.98±1.50 to 3.12±1.49 across non severe and severe categories. This parameter was insignificant on statistical analysis (P=0.67).NLR across the groups was 6.23±5.64 and varied from 3.70±2.65 to 9.99±6.76 among non severe and severe categories respectively (P<0.001).
Overall LMR across all patients was 8.39±7.62. It was 11.03±8.56 in the non severe group and 4.47±3.23 in the severe one with P<0.001, and hence statistically significant (P<0.001).PLR was also found to be significant on statistical analysis in this study, with a P value of 0.023 on analysis across the groups. The values ranged from 114.99±110.27 to 412.68±889.47 across the non severe and severe categories.
Leukocyte counts, NLR; absolute neutrophil and lymphocyte counts and platelet count were found to be useful indicators to differentiate between non severe and severe groups with P<0.001 for each of them by Shang et al[6]. Feng et al in their systematic review and metta analysis spanning 18 studies, with 758 cases in the severe group and 1,688 cases in non-severe group observed a significant increase in number of neutrophils in severe COVID-19 group. Likewise, they studied lymphocytes in 27 studies with 4,480 cases. They reported that lymphocyte count was reduced in severe disease as against non-severe patients and also severe patients have a higher NLR compared with the non-severe group.(8)Higher age and NLR have also been flagged as independent indicators of poor clinical outcome by Yang et al[9]. In a study to identify severe from moderate cases of COVID-19 using combined hematology parameters, NLR, PLR, RDW-CV and RDW-SD in the severe category were significantly higher than the moderate group with P<0.05. On the other hand, Lymphocyte count, Eosinophil count, High Flow Cell percentage, RBC count, Hemoglobin and Hematocrit in the severe group were found to be significantly lesser than those in the moderate category with P<0.05[7]. In the present study, CRP was found to correlate significantly with the severity of groups (P<0.001), with the mean CRP values being 9.64±11.91 mg/L& 114.03±96.90 mg/L in the non severe and severe groups respectively.
C-reactive protein (CRP), being an acute-phase reactant, is increased in inflammatory states. It is also known to play a crucial role in infection protection, warding off autoimmunity, and regulating inflammation. In a systematic review and meta analysis on Immune-inflammatory parameters in COVID-19 cases by Feng et al, CRP was analysed in 20 studies, thereby covering 2,591 COVID-19 patients. They surmised that severe patients have higher CRP than non-severe patients. Also, it was reported that increased CRP was significantly associated with increased disease severity.(8)We had twenty four patients with co-morbidities, of which six were in the non severe group and eighteen in the severe group. All the comorbidities were clubbed together for statistical analysis. The association of comorbidities with clinical severity was studied and the difference among the groups was statistically significant (P<0.001).**Guan et al reported** comorbidities in a quarter of their cases and concluded that they are associated with unfavourable clinical outcomes.(10)Heart disease was found to be a co morbidity which was significantly found to affect disease severity in Covid 19 by Shang et al(6)We analysed

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the optimal cut-off values for NLR, PLR, LMR and CRP was calculated by the ROC analysis, and the ROC curves were presented in Fig. 1. In Fig. 1, areas under the curve (AUC) of age, WBC, NLR, CRP, LMR and PLR were 0.759, 0.775, 0.848, 0.960, 0.202, and 0.772. LMR could not be used as a potential diagnostic biomarker for subsequent analysis because its AUC was less than 0.50. In the present study, ROC curves highlighted the following: For NLR, area under the curve(AUC) was 0.848 (95% CI, 0.766 - 0.931), with an optimal cut off of 4.63. Sensitivity was 0.787, and specificity was 0.755. For PLR the area under the curve (AUC) was 0.772 (95% CI, 0.666 – 0.879), the sensitivity was 0.636, and the specificity was 0.857. The optimum cut off of Age, WBC count, and CRP were 37.5 years, 9650 cells/mm3 and 28.8 mg/L, respectively in our study.Yang et al in their study on diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients, on binary logistic analysis found increased NLR to have a hazard risk of 2.46, with a 95% confidence interval ranging from 1.98–4.57. They also reported age to be a risk factor with a hazard risk of 2.52 and 95% CI from1.65–4.83. These were found to be independent factors for poor clinical outcome of COVID-19. Among the parameters studied- including lymphocyte-to-monocyte ratio, platelet-to-lymphocyte ratio, and C-reactive protein, they surmised that NLR had the largest area under the curve(0.841) with highest sensitivity (88%) and specificity (63.6%). They surmised that higher age and NLR are independent indicators for indicating poor prognosis in Covid 19 infection[9].

Conclusion

The present study has re-emphasized the prognostic and predictive significance of routine hematological investigations and CRP in Covid -19 patients.Awareness regarding the same has had the potential of close clinical monitoring of the high risk group and planning aggressive treatment protocols for better clinical outcomes.

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