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Original Article

Association of Hematologic biomarkers and their combinations with disease severity and mortality in COVID-19- an Indian perspective

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Abstract: Background: COVID-19 is a systemic viral infection with a significant impact on the hematopoietic system, hemostasis as well as immune system. It would be of utmost importance to explore if the most routinely used tests could serve as an aid in determining patient's clinical status or predicting severity of the disease. Methods: A prospective cross-sectional study was conducted on 506 Covid-19 positive patients and 200 controls over a period of two months (June and July 2020). The cases were sub-classified based on disease severity into mild to moderate (n=337), severe (n=118) and very severe (n=51) and based on survivor status into survivors (n=473) and non-survivors (n=33). Results: There were statistically significant differences in WBC count, Absolute neutrophil count (ANC), Absolute lymphocyte count (ALC), absolute monocyte count (AMC), neutrophil–lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), monocyte-lymphocyte ratio (MLR) Red blood cell distribution width (RDW-SD) and RDW CV between covid cases vs controls; among the clinical subgroups and among the survivors and non-survivors. There was a significant strong positive correlation between various parameters, that is, NLR and MLR (r: 0.852, P=0), MPV and PDW (r: 0.912, P=0), MPV and PLCR (r: 0.956, P=0), PDW and PLCR (r: 0.893, P=0). NLR (AUC: 0.676, P=0) was the best single parameter and NLR+RDW-CV was best combination parameter as per area under curve (0.871) of ROC to distinguish severe from mild to moderate disease. Conclusions: Leucocytosis, neutrophilia, lymphopenia and monocytosis were characteristic findings in covid cases while NLR and NLR+RDW-CV emerged as the most effective single and combination CBC parameters in distinguishing mild to moderate and severe cases respectively.

Keywords: COVID-19, hematological indices, NLR, PLR, MLR, RDW-SD, RDW-CV

Introduction

The coronavirus disease popularly known as the COVID-19 disease was first reported in Wuhan, Hubei in China in December 2019 and has rapidly evolved from an epidemic outbreak into a global pandemic infecting more than thirteen million individuals across the world [1]. SARS-COV2 virus has been implicated as the causative agent [2]. First covid-19 case in India was reported from Kerala on 30th Jan 2020 with a positive travel history to Wuhan.

Covid-19 is a systemic viral infection with a significant impact on the hematopoietic system, hemostasis as well as immune system. Recent preliminary data following the Covid-19 outbreak indicated an association of complete blood count (CBC) parameters [3] and coagulation profile (increased D-Dimer, fibrinogen and FDP) with disease progression [4, 5].

Red blood cell distribution width (RDW) has recently gained importance as an inflammatory marker in many diseases [6]. Among the platelet parameters, mean platelet volume (MPV) is a reflection of the average size of platelets and is most extensively researched and has been found to increase in myocardial infarction and coronary artery disease [7]. There is a dearth of literature on these parameters in Covid-19 [8].

The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) have both emerged

as good indicators of subclinical systemic inflammation in a variety of diseases such as cancer, cardiovascular diseases, and autoimmune inflammatory diseases [9]. NLR and PLR have an advantage of being relatively stable compared to individual blood cell parameters. These have been evaluated by various studies in the literature in Covid-19 indicating an association with severity of disease [10].

In the current COVID-19 scenario, it would be of utmost importance to explore if the most routine and cost-effective tests like CBC could serve as an aid in determining patient's clinical status or predicting severity of the disease leading to a better and judicious allocation of medical resources especially in resource-poor settings. Moreover, mortality may also be reduced by early and timely clinical intervention.

Material and methods

The present study was a prospective cross-sectional study of all positive COVID-19 patients admitted to ESIC MC and Hospital, Faridabad over a period of two months (June to July 2020). The study was approved by Institutional Ethics Committee and written informed consent was obtained. Detailed clinical and demographical data of the study subjects was documented.

Sample collection

The venous blood samples were collected in EDTA vacutainer on day 3-6 of admission to the isolation ward/intensive care unit (ICU) for Complete Blood Count (CBC) and ESR. CBC was analyzed on fully automated 6 part hematology analyzer (Sysmex XN 1000) while ESR was analyzed on VESMATIC CUBE 80 analyzer. The ratios were calculated from hematological parameters. The sample collection, processing and discarding was done using proper PPE as per standard protocols.

Inclusion criteria

All healthy adult individuals with no symptoms were taken as controls. All patients above 18 years of age who tested positive on real time reverse transcriptase polymerase chain reaction (RT-PCR) were considered as Covid-19 positive cases irrespective of clinical symptoms (asymptomatic, mild, moderate or severe).

Based on clinical severity, the cases were divided into three groups: mild to moderate, severe and very severe.

Operational definitions

Mild to moderate: A patient who presented with fever, sore throat, cough etc. not meeting the criteria for severe/very severe disease.

Severe COVID-19: It was defined as possessing one of the following criteria: 1) Respiratory distress with respiratory rate more than 30 times/min; 2) Oxygen saturation ≤ 93% in resting state; 3) PaO2/FiO2 ≤ 300 mmHg (1 mmHg = 0.133 kPa).

Very severe COVID-19: It was defined as possessing one of the following criteria: 1) Respiratory failure in need of mechanical ventilation; 2) Shock 3) Other organ dysfunction [9].

The patients were also divided into survivors and non-survivors based on their survival status.

Exclusion criteria

The patients with history of thromboembolic disorders, cardiovascular disease, inflammatory bowel disease, hematological disorders, trauma or surgeries in last six months or bedridden patients, pregnant females or drugs known to affect the coagulation profile/platelets, kidney or liver disease, neoplasms, were excluded from the study.

The CBC parameters evaluated include: Hemoglobin (Hb), white blood cell count (WBC), hematocrit (Hct), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW-CV, RDW-SD), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), absolute eosinophil count (AEC), platelet count, platelet parameters-MPV, platelet distribution width (PDW), plateletcrit (PCT), platelet large cell ratio (PLCR). Ratios derived from these parameters, that is, NLR, PLR, MLR were also assessed in both cases and controls.

Data and statistical analysis

The data collected for 506 cases and 200 controls was entered in Microsoft excel sheet

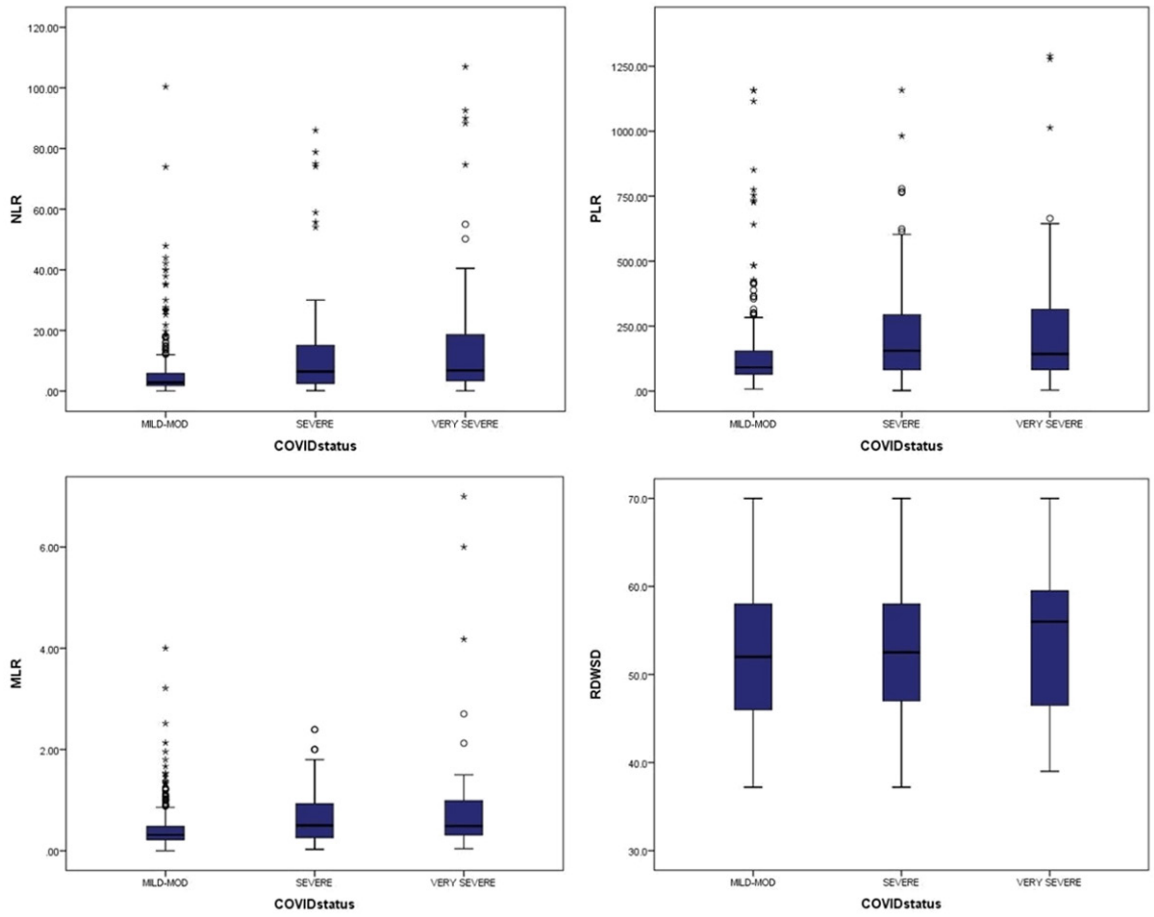

Figure 1. The comparison of complete blood count parameters with significant differences between mild to moderate, severe and very severe groups through box plots.

and analyzed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). The continuous data was presented as mean, median and box plots. The continuous variables in the two groups i.e., cases and controls and the three groups viz., moderate, severe and very severe were subjected to test of normality. The non-normally distributed variables in two groups (cases and controls or survivor and non-survivor) were compared using Mann Whitney U test; among three groups (Moderate, Severe and Very severe) were compared using Kruskal Walis test and normally distributed variables were analysed using ANOVA test. The variables grouped into survivor and non-survivor groups were normally distributed and t-test was applied to test the statistical difference in two groups. The categorical data was presented as proportions. Pearson's correlation coefficient was calculated for studying association between laboratory parameters in COVID positive cases. Receiver operation characteristic curve (ROC curve) was used to analyze the efficiency of parameters in predicting disease severity. The severe and very severe cases as defined above were taken as primary outcome for calculating area under the curve (AUC) for parameters. Two parameters along with age and sex were subjected to binary logistic regression model with severity as outcome and a new variable was generated as predicted probability. *P* value <0.05 was considered statistically significant.

Results

Demographic characteristics of study population

A total of 506 COVID-19 positive patients were enrolled for the study while 200 patients served as controls. Classified on the basis of disease severity, the study population was divided into: mild to moderate (n=337), severe

Table 1. Comparative analysis of clinical and hematological parameters in COVID-19 patients and controls

	COVID (n=506)	Control (n=200)	*p* value
Age	42.34±16.06	48.10±10.39	<0.0001*
M:F ratio	1.69:1	1.5:1	-
Mortality	6.5%	-	-
Hb	11.57±2.29	14.53±1.15	<0.0001*
Hct	37.47±12.5	37.37±7.36	0.86*
MCV	90.26±12.57	91.10±9.92	0.41*
MCH	28.12±3.39	28.15±3.39	0.90*
MCHC	30.84±1.56	30.88±1.45	0.77*
RDW CV	16.15±2.68	13.59±0.98	<0.0001*
WBC	10.99±7.87	7.21±0.85	<0.0001*
ANC	7.96±6.28	4.95±1.37	<0.0001**
ALC	1.93±2.17	2.35±0.59	0.001*
AMC	0.71±0.61	0.62±0.61	0.008**
AEC	0.16±0.42	0.22±0.23	<0.0001**
NLR	10.35±26.4	1.76±0.56	<0.0001**
PLR	200.3±3.67	87.60±20.53	<0.0001*
MLR	0.58±0.62	0.28±0.26	<0.0001**
Platelet	194.62±106.72	205.47±45.01	0.06*
PDW	16.12±3.6	15.69±2.30	0.03*
MPV	12.15±1.27	12.18±1.27	<0.0001*
PLCR	41.43±9.27	40.93±10.47	0.56*
PCT	0.23±0.08	0.31±0.07	<0.0001*
ESR	25.79±7.38	10.97±3.87	<0.0001*

*independent "t" test, **Mann Whitney U test, *p* value less than 0.05 is considered statistically significant. The continuous variables in the two groups i.e., cases and controls were subjected to test of normality. The non-normally distributed variables in two groups (cases and controls) was compared using Mann Whitney U test; and normally distributed variables were analysed using t test. Hemoglobin (Hb), white blood cell count (WBC), hematocrit (Hct), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW-CV, RDW-SD), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), absolute eosinophil count (AEC), Mean platelet Volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet large cell ratio (PLCR), Erythrocyte sedimentation rate (ESR).

(n=118) and very severe groups (n=51). The mean age of the patients was 42 years with a male to female ratio of 1.69:1. Out of the 506 cases, 33 patients expired during the course of treatment, the mortality rate being 6.5%.

Hematologic profile of COVID-19 patients vs controls

Leucocytosis, neutrophilia, lymphopenia and monocytosis were characteristic findings observed in COVID-19 patients. On comparing COVID-19 patients (n=506) with controls (n=200), there was a statistically significant difference in Hemoglobin (hb), Red Blood Cell (RBC) count, MCH, MCHC, RDW SD and RDW CV. Among the white blood cell (WBC) parameters, WBC count, Absolute neutrophil count (ANC), Absolute lymphocyte count (ALC), absolute monocyte count (AMC), NLR, PLR while among the platelet parameters, only MPV and plateletcrit (PCT) were found to be significantly different between the two groups.

Hematologic profile of subcategories of COVID-19 patients based on disease severity

When COVID-19 patients were further classified into mild to moderate, severe and very severe categories, statistically significant differences were observed in RDW SD, RDW CV, WBC count, ANC, AMC, NLR, PLR, MLR, platelet count, MPV and PDW. The ratios NLR, PLR and MLR were found to be increasing uniformly from mild to moderate to severe to very severe groups. Box plots depicting differences in hematological parameters between various clinical categories of COVID-19 are shown in **Figure 1**. Comparison of clinical and hematological parameters between COVID-19 patients and controls and between the clinical subcategories of COVID-19 disease is shown in **Tables 1** and **2**.

Hematologic profile of subcategories of Covid patients based on survivor status

Based on survival status when COVID-19 patients were further divided into survivors and non-survivors, statistically significant differences were observed in RDW SD, RDW CV, WBC count, ANC, ALC, AMC, NLR, PLR, MLR, platelet count, MPV, PLCR and PDW (**Table 3**). All the ratios and RDW were found to be elevated in non-survivors while platelet count was reduced in on survivors compared to survivors.

Correlation between hematological parameters

There was a significant strong positive correlation between various parameters, that is, NLR and MLR (r: 0.852, P=0), MPV and PDW (r: 0.912, P=0), MPV and PLCR (r: 0.956, P=0),

Table 2. Comparative analysis of Clinical and Hematological parameters in subcategories of COVID-19 patients based on disease severity

	Very severe (n=51)	Severe (n=118)	Moderate (n=337)	*p* value
Age	64.06±11.05	52.38±14.86	35.3±11.8	-
M:F ratio	3.25:1	2.37:1	1.39:1	-
Mortality	60.8%	1.7%	0%	-
Hb	12.01±2.20	11.58±2.03	11.5±2.39	0.526*
Hct	38.95±6.78	33.73±6.19	37.3±7.27	0.350*
RBC	4.3±0.91	4.19±1.21	4.16±0.89	0.630**
MCV	91.86±6.88	89.64±14.17	90.24±12.02	0.806*
MCH	28.4±3.9	28.43±3	27.96±3.43	0.555*
MCHC	30.96±1.53	31.03±1.55	30.7±1.56	0.151*
RDW SD	56.31±8.09	52.55±6.7	51.78±7.8	<0.0001**
RDW CV	16.19±2.51	15.75±2.88	15.30±2.63	0.076*
WBC	16.75±11.93	12.05±7.39	9.74±6.76	<0.0001*
ANC	12.31±7.24	9.4±6.69	6.79±5.54	<0.0001*
ALC	2.53±5.31	1.79±2.08	1.89±1.2	<0.0001*
AMC	0.93±0.85	0.76±0.59	0.65±0.56	0.001*
AEC	0.1±0.19	0.19±0.22	0.16±0.28	<0.0001*
NLR	19.58±27	17.36±22.56	7.43±30.02	<0.0001*
PLR	260.71±290.65	234.38±242	149.42±272	<0.0001*
MLR	0.96±1.33	0.66±0.53	0.49±1.18	<0.0001*
PLATELET	212.9±97.16	218±124	183±99.88	0.007*
PDW	17.43±3.78	16.24±3.51	15.99±3.6	0.012*
MPV	12.55±1.2	12.22±1.24	12.09±1.3	0.050**
PLCR	42.94±8.93	41.67±8.8	41.12±9.48	0.406**
PCT	0.23±0.08	0.23±0.07	0.24±0.089	0.278**
ESR	29.56±5.42	24.34±5.86	22.8±5.87	<0.0001**

*Kruskal Wallis test, **ANOVA, *p* value less than 0.05 is considered statistically significant. The continuous variables in the three groups viz., moderate, severe and very severe were subjected to test of normality. The non normally distributed variables in three groups (Moderate, Severe and Very severe) were compared using Kruskal Walis test and normally distributed variables were analysed using ANOVA test.

PDW and PLCR (r: 0.893, P=0). A significant moderate correlation was observed between WBC and NLR (r: 0.435, P=0), WBC and MLR (r: 0.405, P=0), MPV and PCT (r: -0.440, P=0), PDW and PCT (r: 0.468, P=0), and PCT and PLCR (r: -0.443, P=0). The correlation of various parameters with each other and with severity indices is depicted in **Table 4**.

The results showed that NLR (AUC: 0.676, P=0) was the best single parameter as per area under curve of ROC in distinguishing mild to moderate and severe cases (severe and very severe). NLR was followed by WBC count (AUC: 0.662, P=0), PLR (AUC: 0.654, P=0), MLR (AUC: 0.654, P=0), RDW-CV (AUC: 0.549, P=0.07) and RDW-SD (AUC: 0.535, P=0.203) (**Figure 2**).

The combined parameters were assessed for the diagnostic efficacy analysis in the differentiation between the severe and the mild to moderate groups. The NLR and RDW-CV combination had the best diagnostic efficiency (AUC=0.871), followed by NLR and RDW-SD (AUC= 0.861), NLR and PLR (AUC= 0.859) and NLR and MLR (AUC= 0.858) (**Table 5** and **Figure 2**).

Discussion

COVID-19 is a systemic multi organ disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a beta-type RNA coronavirus [11]. It is more contagious than either SARS-CoV (in 2003) or MERS-CoV (in 2012) but has lower case fatality [12-14]. The virus primarily attacks the lung, causing drastic lung injury and acute respiratory distress syndrome (ARDS) in severe cases which could be fatal [15]. The postulated pathogenesis states that the virus binds to the ACE2 receptor and gains entry into the alveolar epithelial cells [16], inciting a cytokine storm due to immune activation resulting in inflammation and tissue damage [15].

The mean age of covid-19 cases was 42 years with increased severity in older patients similar to others studies in the literature [9, 11, 12] highlighting the fact that the disease is significantly severe in elderly and those with co-morbidities. The global mortality rate from COVID-19 is 4.39%, ranging from 1.71% in Africa to 6.9% in Europe [1]. South East Asia has a mortality rate of 2.5% [1] while in the present study it was 6.5% as only the hospitalized patients were taken into account not the home isolated ones.

Table 3. Comparative analysis of Hematological parameters in COVID-19 patients based on survivor status

	SURVIVOR (n=473)	NON SURVIVOR (n=33)	*p* Value
Hb	11.57±2.29	11.77±2.23	0.627*
Hct	37.47±6.99	38.3±7.15	0.510*
RBC	4.16±0.97	4.34±1.02	0.305*
MCV	90.28±12.5	90.03±13.81	0.912*
MCH	28.14±3.33	27.76±4.22	0.534*
MCHC	30.84±1.56	30.88±1.52	0.887*
RDW SD	52.04±7.45	54.63±9.26	0.050*
RDW CV	16.16±2.72	17.13±2.02	0.045*
WBC	10.99±7.86	18.67±12.14	<0.0001*
ANC	7.96±6.28	14.12±7.38	<0.0001**
ALC	2.12±2.18	1.29±1.03	0.097**
AMC	0.71±0.61	1.04±0.99	0.006**
AEC	0.16±0.26	0.10±0.21	0.001**
NLR	10.97±27.17	23.07±30.35	<0.0001**
PLR	147.97±132	211.77±153	0.031**
MLR	0.58±1.09	1.07±1.52	0.001**
Platelet	193.99±107.31	151±43.20	0.022*
PDW	16.08±3.64	17.37±3.00	0.047*
MPV	12.14±1.29	12.88±0.99	0.002*
PLCR	41.25±9.36	44.95±7.63	0.027*
PCT	0.24±0.09	0.22±0.07	0.212*
ESR	25.79±7.38	26.76±7.29	0.186*

*independent "t" test, ** Mann Whitney U test, *p* value less than 0.05 is considered as statistically significant. The continuous variables in the two groups i.e., survivor and non survivor were subjected to test of normality. The non normally distributed variables in two groups (survivor and non survivor) was compared using Mann Whitney U test and normally distributed variables were compared using t test.

The index study showed a downward trend in hemoglobin in covid-19 patients compared to controls as well as among the subgroups based on clinical severity, which is in accordance with several authors [9, 11, 12]. This could be on account of SARS-CoV-2 damaging the kidney tissue which is rich in ACE2-receptor [17] and elevated inflammatory factors, leading to reduced erythropoiesis and increased destruction of RBC, contributing to anemia.

The inflammatory cytokines may induce profound alterations in the behaviour of hematopoietic cells, mainly neutrophils, lymphocytes and monocytes. WBC and neutrophil counts were significantly higher in covid-19 patients, especially the patients with severe disease. These results are consistent with the findings of Chen et al [2], Wang et al [12], Wu et al [18], Huang et al [19], Ding et al [20] etc. This may be linked to persistent infection and prolonged hypoxia leading to release of more granulocytes as a compensatory mechanism by the bone marrow. Li et al [21] have reported superimposed bacterial pneumonia in some non-survivors with COVID-19.

Among all CBC parameters, lymphocyte counts showed the most significant and consistent trend and might reflect the progression of disease. Guan et al [15] studied a large cohort of 1,099 Covid-19 patients and observed lymphopenia in 82.1% patients and ever since has been documented by most of the studies [3, 7, 11, 12, 19, 20, 22] on Covid-19 including the current one. Tan et al [22] established a Time-LYM% model (TLM) for disease classification and prognosis prediction. There could be potential mechanisms to explain lymphopenia: (1) the virus might directly target lymphocytes, which express the coronavirus receptor ACE 2 resulting in lymphocyte death, (2) the virus could directly destroy lymphatic organs like thymus and spleen, (3) cytokines (tumour necrosis factor (TNF) α, interleukin (IL)-6, etc.) could induce lymphocyte deficiency, (4) molecules like lactic acid produced by metabolic disorders could inhibit lymphocytes [22].

In acute lung infection caused by virus, usually eosinophils tend to accumulate in infected tissues to resist virus infection, therefore eosinopenia is found in peripheral blood [23]. Eosinopenia was encountered upon comparison of covid-19 patients with controls and non-survivors with survivors, however, this phenomenon was not statistically significant.

The platelet count is a reflection of platelet turnover, serving as a sensitive indicator of severity of disease and is very helpful in monitoring patients with aggressive viral illness. In the current study, the difference in platelet counts between cases and controls were not statistically significant. On the contrary, Zhao et al [24], Lippi et al [25] and Liu et al [26] with

Table 4. Correlations between the hematological markers of COVID-19 patients

		WBC	NLR	PLR	MLR	PLT	MPV	PDW	PCT	PLCR	RDW SD	RDW CV
WBC	r	1	0.44	0.15	0.41	0.11	0.05	0.05	0.05	0.05	-0.02	0.02
	p		<0.0001	0.001	<0.0001	0.010	0.27	0.29	0.25	0.27	0.71	0.69
NLR	r	0.44	1	0.52	0.85	0.01	0.03	0.01	-0.01	0.02	-0.04	-0.01
	p	<0.0001		<0.0001	<0.0001	0.87	0.53	0.86	0.80	0.68	0.43	0.87
PLR	r	0.15	0.52	1	0.33	0.33	0.06	0.04	-0.002	0.05	0.03	0.002
	p	.001	<0.0001		<0.0001	<0.0001	0.18	0.38	0.96	0.22	0.54	0.96
MLR	r	0.41	0.85	0.33	1	0.05	0.05	0.04	-0.02	0.04	-0.08	0.02
	p	<0.0001	<0.0001	<0.0001		0.27	0.23	0.37	0.69	0.39	0.09	0.67
PLT	r	0.11	0.01	0.33	0.05	1	0.04	0.02	0.05	0.04	0.08	0.07
	p	0.01	0.87	<0.0001	0.27		0.44	0.69	0.25	0.34	0.07	0.10
MPV	r	0.05	0.03	0.06	0.05	0.04	1	0.91	-0.44	0.96	0.01	0.02
	p	0.27	0.53	0.18	0.23	0.44		<0.0001	<0.0001	<0.0001	0.82	0.72
PDW	r	0.05	0.01	0.04	0.04	0.02	0.91	1	-0.47	0.89	0.02	.045
	p	0.29	0.86	0.38	0.37	0.69	<0.0001		<0.0001	<0.0001	0.69	.317
PCT	r	0.05	-0.01	-0.002	-0.02	0.05	-0.44	-0.47	1	-0.44	-0.001	-.073
	p	0.25	0.80	0.96	0.69	0.25	<0.0001	<0.0001		<0.0001	0.98	.100
PLCR	r	0.05	0.02	0.05	0.04	0.04	0.96	0.89	-0.44	1	-0.02	.024
	p	0.27	0.68	0.22	0.39	0.34	<0.0001	<0.0001	<0.0001		0.73	.593
RDWSD	r	-.017	-0.34	0.03	-0.08	0.08	0.01	0.02	-0.001	-0.02	1	0.06
	p	0.71	0.43	0.54	0.09	0.07	0.82	0.69	0.98	0.73		0.17
RDWCV	r	.017	-0.01	0.002	0.02	0.07	0.02	0.05	-0.07	0.02	0.06	1
	p	.696	0.86	0.96	0.67	0.10	0.72	0.32	0.10	0.59	0.17	

*r: correlation coefficient, *p* value less than 0.05 is considered statistically significant. Pearson's correlation coefficient was calculated for studying association between laboratory parameters in COVID positive.

poor prognosis in covid-19 patients documented that thrombocytopenia was associated with enhanced risk of severe COVID-19 and mortality. The probable causes of platelet changes in COVID-19 patients might be a direct invasion of hematopoietic cells or bone marrow stromal cells by corona virus or the lung injury prevents release of platelets from mature megakaryocytes as lung might be one of the organs where it occurs [26, 27].

Foy et al [28] similar to our findings, observed that elevated RDW at diagnosis and an increase in RDW during admission are both associated with increased mortality risk for adult COVID-19 patients.

The derived inflammation indices like NLR, PLR and MLR reflect the hematological scenario in a much better way compared to the individual cell counts as speculated by several authors [8, 11, 12, 20, 29-32] in judging the severity of COVID-19. NLR is a widely used biomarker for assessment of the severity of bacterial infection. Our results showed that NLR was elevated in patients with severe disease, which is consistent with findings of most of the authors [8, 12, 20, 30]. Qu et al [32] advocated active intervention when PLR is > 126.7 to prevent further deterioration of the disease. They inferred that PLR indicates the degree of cytokine storm and may serve as a novel marker for monitoring disease progression in covid-19 patients.

Significant differences were noted in MLR among different subgroups based on disease severity. Our findings were supported by Sun et al [11] and Lissoni et al [33].

Wang et al [12] studied a variety of combination parameters and concluded that NLR & RDW-SD is the best hematology index which can aid in predicting the severity of COVID-19 patients, followed by the fitting parameter NLR & RDW-CV. Similarly, we found that the combined parameter NLR and RDW-CV had the best diagnostic efficiency (AUC=0.871) in predicting severe disease.

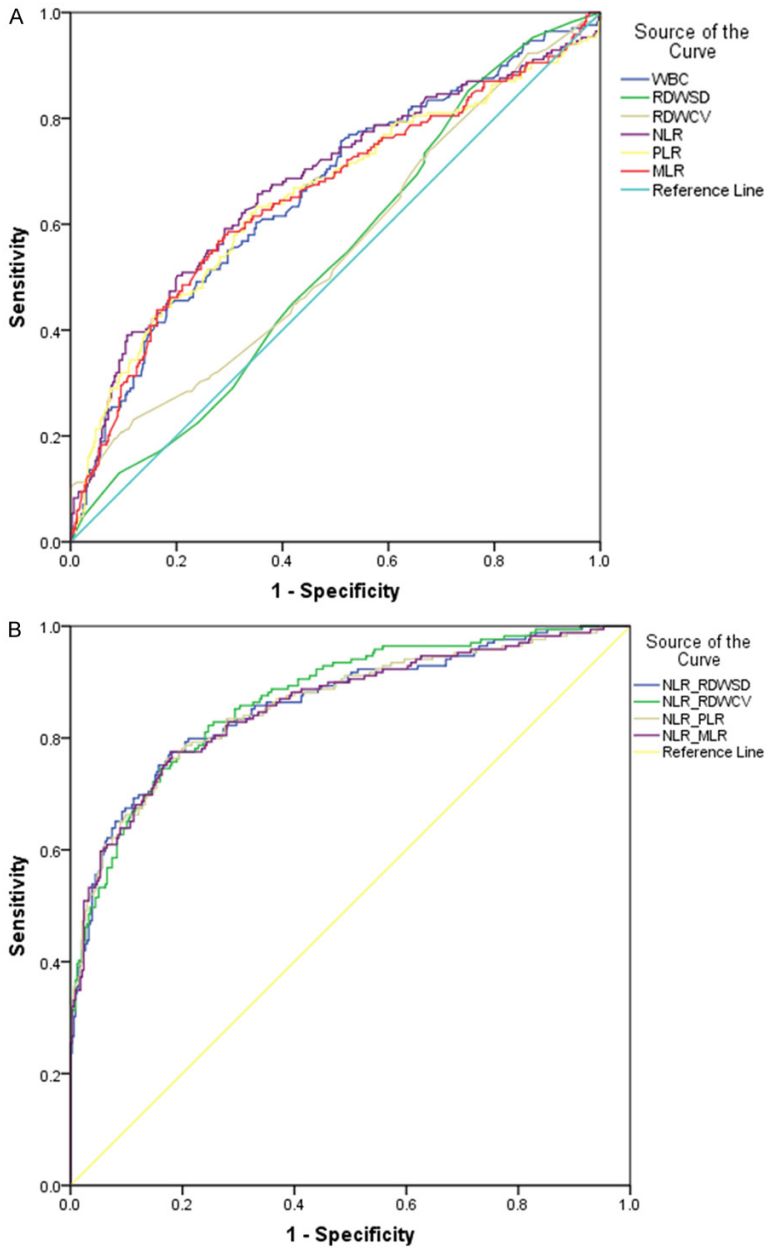

Figure 2. A: ROC plot of single parameters (WBC count, NLR, PLR, MLR, RDW-SD, RDW-CV). * ROC Analysis using single parameters in the diagnosis of severe cases of Covid-19. The positive sample is the CBC results of severe and very severe cases while the negative sample is the results of mild to moderate cases. B: ROC plot using combination parameters (NLR and RDW-SD, NLR and RDW-CV, NLR and PLR, NLR and MLR). * ROC Analysis using combined parameters in the diagnosis of severe cases of Covid-19. The positive sample is the CBC results of severe and very severe cases while the negative sample is the results of mild to moderate cases.

On extensive literary review, we came across only a single Indian pilot study by Tiwari et al [34] (n=32) in COVID-19 patients with similar observations as in global literature. The present study is the first Indian study catering to a large patient cohort and providing important insights into the hematological changes induced by COVID-19 from the Indian perspective.

The current study had certain limitations-First, the possibility of residual unmeasured potential confounding factors cannot be excluded, second the reported associations between hematological parameters and severity of disease/mortality cannot be concluded as causal as it's a cross sectional study. Large multicentre cohort studies are needed to overcome these limitations.

Conclusions

As Covid-19 pandemic has approached massive proportions, our best bet is to ensure meticulous allocation of resources to provide effective treatment for critical patients utilizing early warning markers, thereby reducing the overall mortality. On evaluating the most routine and cost effective tests like CBC, leucocytosis, neutrophilia, lymphopenia and monocytosis were characteristic findings observed in COVID-19 patients. NLR (AUC: 0.676, P=0) emerged as the single best parameter and NLR+RDW-CV was the best combination parameter as per area under curve (0.871) of ROC to distinguish severe from mild to moderate disease. Nevertheless, our results provided important insights into this topic which may help the clinicians in predicting the disease severity and take effective treatment measures well in advance and also aid in controlling the epidemic effectively especially in developing countries like India decreasing the burden on the existing healthcare infrastructure.

Table 5. Area under the curve for various hematological parameters in severe and very severe versus moderate COVID-19 patients

Variable	Area under curve	*p* value	95% Confidence Interval	
			Lower Bound	Upper Bound
WBC	.662	.000	.610	.713
RDWSD	.535	.203	.483	.587
RDWCV	.549	.070	.495	.603
NLR	.676	<0.0001	.623	.728
PLR	.654	<0.0001	.600	.707
MLR	.654	<0.0001	.601	.707
NLR and RDWSD	.861	<0.0001	.825	.897
NLR and RDWCV	.871	<0.0001	.838	.904
NLR and PLR	.859	<0.0001	.823	.896
NLR and MLR	.858	<0.0001	.821	.894

The severe and very severe cases were taken as primary outcome for calculating area under the curve (AUC) for parameters. A new variable was generated by combining two parameters along with age and sex and were then subjected to binary logistic regression model with severity as outcome. The new variable was generated in form of predicted probability from the model.

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Disclosure of conflict of interest

None.

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