Research Article

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Evaluation of prognostic markers in patients infected with SARS-CoV-2

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Abstract: Prognostic markers are the biomarkers used to measure the disease progression and patient outcome regardless of treatment in coronavirus disease 2019 (COVID-19). This study aimed to analyze laboratory parameters as prognostic markers for the early identification of disease severity. In this study, 165 patients attending Sukraraj Tropical and Infectious Disease Hospital with COVID-19 were enrolled and divided into severe and non-severe groups. The demographic data, underlying co-morbidities, and laboratory findings were analyzed and compared between severe and non-severe cases. The correlation between the disease criticality and laboratory parameters was analyzed. Cut-off values of parameters for severe patients were speculated through the receiver operating characteristics (ROC) curve, and regression analysis was performed to determine the risk factors. Patients with severe COVID-19 infection had significantly higher absolute neutrophil count, neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), ferritin, positive carbohydrate reactive protein (CRP), glucose, urea, creatinine, and aspartate aminotransferase, while lower absolute lymphocyte count, absolute eosinophil count (AEC), and red blood cell count in comparison to non-severe infection. ROC analysis gave a cut-off value (sensitivity, specificity) of age, AEC, NLR, PLR, and ferritin as 47.5 years (70.2, 64.7%), 335 cells/mm^3 (74, 67%) 3.3 (68.4, 63.7%), 129 (77.2, 51%), and 241 ng/mL (74.0%, 65.0%) respectively. Risk factor analysis showed higher age, low AEC, high ferritin, and positive CRP as independent risk factors associated with severe COVID-19 infection. Hematological and inflammatory markers, including novel NLR and PLR, should be assessed to aid clinicians in the early identification of severe cases, prioritization of cases, and effective management to decrease the mortality of COVID-19 patients.

Keywords: COVID-19, severity, NLR, PLR, CRP

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1 Introduction

Coronaviruses are enveloped, positive sense, single-stranded RNA viruses with a comparatively larger genome (30 kb), which belong to the order Nidovirales family Coronaviridae and subfamily Coronavirinae [1]. In late 2019, the very first case of coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was reported from Wuhan, China, and due to its alarming level of transmission and severity to public health, World health organization declared a global pandemic on March 11, 2020 [2].

COVID-19 infection could start with flu-like symptoms; although it could be asymptomatic, about 15% of the patients are complicated with disease severity [3]. COVID-19 patients typically present with fever, myalgia, respiratory symptoms such as nonproductive cough and dyspnea, decreased lymphocyte counts, and radiographic evidence of bilateral interstitial pneumonia; clinical presentation ranges from mild to critical case, requiring intensive care unit (ICU) admission [4]. COVID-19-associated mortality varies broadly according to geographical areas, patient demographic characteristics, and other comorbidities [5–9].

The major finding of immunopathology in COVID-19 is the cytokine storm. The rapid replication of the virus in epithelial and endothelial cells results in the development of significant numbers of proinflammatory cytokines and chemokine that eventually leads to acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF) [10]. Critical cases quickly progress to complications like ARDS, septic shock, metabolic acidosis, coagulopathy, and MOF [11,12].

Based on clinical symptoms and laboratory findings, the patient can be classified as a mild, moderate, severe, and critical case [13,14]. Among them, 81% account for mild/moderate cases, while 14 and 5% for severe and critical cases [15,16]. Almost 20% of hospitalized patients require an ICU admission, with a mortality rate (61.5%) [12,17].

Different hematological parameters (complete blood count, erythrocyte sedimentation rate, coagulation profile, D-dimer), biochemical parameters: liver function test (LFT), renal function test (RFT), electrolytes, cardiac enzymes, procalcitonin, lactate dehydrogenase, and inflammatory markers: carbohydrate reactive protein (CRP), ferritin as prognostic markers can be used to measure the disease progression to severity and patient outcome regardless of treatment [18].

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are the established inflammatory markers and are readily calculated with hemograms, an inexpensive tool for the early stratification of risk groups and treatment. The diagnostic and prognostic value of NLR is established in cardiovascular disease (CVD), thyroiditis [19], functional bowel conditions [20], and COVID-19 infection [21]. PLR is another novel marker of inflammation, and elevated PLR has been reported in thyroid conditions [22], cancer [23], diabetes mellitus (DM) [24], and irritable bowel disease [20]. CRP is an inflammatory marker increased in inflammatory conditions such as thyroiditis [25], hepatitis C [26], and type 2 DM [27].

Thus, this study aims to identify the role of these biomarkers as a predictor of severity and clinical outcomes during disease, which could help in the early identification of the risk group patients and better patient management.

2 Methods

This laboratory-based cross-sectional study was conducted for the period of 3 months (November 2020 to January 2021) in Sukraraj Tropical and Infectious Disease Hospital (STIDH), Kathmandu, Nepal, in collaboration with Manmohan Memorial Institute of Health Sciences (MMIHS), Kathmandu, Nepal.

2.1 Inclusion and exclusion criteria

COVID-19-infected patients confirmed with positive reverse transcriptase polymerase chain reaction (RT-PCR) and presented with clinical symptoms were included in the study, while a repeated sample from the same patient and asymptomatic patients were excluded from the study.

All 165 patients who fulfilled with above-mentioned inclusion criteria were taken with informed & written consent and recorded with their demographic data, co-morbidities, and laboratory parameters using standard performance.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, and institutional policies and in accordance with the tenets of the Helsinki Declaration, and has been approved and has been approved by the Institution Review Committee of Manmohan Memorial Institute of Health Sciences (IRC MMIHS), Kathmandu, Nepal (letter of approval Ref No: MMIHS-IRC 484).

2.2 Experimental protocol

Venous blood samples were collected following standard operating procedures. In addition, whole blood was collected in K2 EDTA vacutainer (BD Vacutainer, USA), Gel and clot activator tube (Hebei Xinle Sci & Tech Co. Ltd, China), and Sodium citrate vacutainer (BD Vacutainer, USA), which was used for hematological parameters, and serum was separated for inflammatory markers and biochemical analysis.

Whole blood samples were analyzed for hematological parameters such as complete blood cell count (CBC) including hemoglobin (Hb), red blood cell count (RBC), hematocrit (Hct), white blood cell count (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), absolute eosinophil count (AEC), absolute basophil count (ABC), Platelets, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) using a coulter counter (Sysmex XN-330, Asia Green, Singapore) in the Department of Hematology, STIDH. NLR and PLR were calculated manually using the data obtained from the coulter counter. In addition, ferritin was estimated quantitatively by nephelometer (mispa-i2, Kerala, India) as per the instructions provided by the reagent manufacturer (AGAPPE DIAGNOSTICS LTD, Kerala, India), and qualitative CRP was performed by following the manufacturer's guideline (Omega diagnostics, UK).

Random blood sugar, LFT including alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, and RFT including urea, creatinine, sodium, and potassium were estimated. All the parameters were analyzed using a fully automated random access clinical chemistry analyzer XL-200 (ERBA, Mannheim, Germany) in the Department of Biochemistry, STIDH.

Diagnosis and clinical classification of COVID-19 according to the new coronavirus pneumonia diagnosis and treatment plan (trial version 7) developed by the National Health Commission of the People's Republic of China are as follows: (1) mild, minor symptoms and imaging show no pneumonia; (2) moderate, with fever, respiratory tract symptoms and imaging show pneumonia; (3) severe, meet any of the following: (a) respiratory distress, respiratory rate $\geq$ 30 beats/min; (b) oxygen saturation $\leq$ 93%; (c) arterial blood oxygen partial pressure $\leq$ 300 mmHg, pulmonary imaging showed that the lesion progressed more than 50% within 24–48 h. (4) critical, one of the following conditions: (a) respiratory failure occurs and requires mechanical ventilation, (b) Shock occurs and (c) ICU admission is required for combined organ failure [28].

2.3 Statistical analysis

Data were collected in Microsoft Excel 2013 and analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The normal distribution was analyzed using the Shapiro-Wilk test. Independent sample *t*-test for normally distributed variable and expressed as mean ± SD, and Mann–Whitney *U* test for non-normally distributed variable expressed as median (Q_1–Q_3) were used to analyze the differences in laboratory parameters between severe and non-severe patients. Categorical variables were presented as numbers (percentage) and compared using the Chi-square test. Receiver-operating characteristics (ROC) curve analysis was used to determine the optimum cut-off points of the parameters. Similarly, a risk estimate was obtained for the severity of the disease. Univariate analysis was done to obtain the association of risk factors for disease severity. Further, the associated covariates were entered in stepwise multivariate risk analysis for adjusted odds ratio using a binary logistic regression model.

3 Results

A total of 165 COVID-19 patients were enrolled, among which 114 (31%) had a severe infection while 51 (69%) had a non-severe infection. Higher age was significantly associated with disease severity ($p < 0.001$). Male and female participants were 124 (75.2) and 41 (24.6%), respectively (Tables 1 and 2).

Table 1: Characteristics of the patients with COVID-19

Variable	Total *N* = 165 *n* (%)	Non-severe *N* = 51 *n* (%)	Severe *N* = 114 *n* (%)	*p*-Value
Age in years				
(Mean ± SD)	53.5 ± 16.5	44.4 ± 14.3	57.5 ± 15.3	**<0.001**[a]
<30	15 (9.1)	11 (21.6)	4 (3.5)	
30–49	54 (32.7)	22 (43.1)	32 (28)	
50–69	64 (38.8)	16 (31.4)	48 (42.1)	
70–89	31 (18.8)	2 (15.7)	29 (25.4)	
>89	1 (0.6)	0 (0)	1 (0.9)	
Gender				
Male	124 (75.2)	38(74.5)	86 (75.4)	0.898[b]
Female	41 (24.6)	13(25.5)	28 (24.6)	

$p < 0.05$ was considered statistically significant.
[a]*p*-value calculated using an independent sample *t* test.
[b]*p*-value calculated using the chi-square test.

Table 2: Hematological and inflammatory markers of a patient with COVID-19

Laboratory parameters Median (IQR)	Total *N* = 165	Disease severity		*p*-Value
		Non-severe *N* = 51	Severe *N* = 114	
Hb (gm/dL)	13.2 (12–14.4)	13.4 (12–15)	13.1 (12.2–14.2)	0.23
RBC (millions/cumm)	4.5 (4.1–5)	4.8 (4.2–5.2)	4.5 (4.1–1.9)	**0.037***
Hct (%)	39 (15, 36–42)	40 (15, 35–42)	39 (15, 36–41)	0.40
WBC ($\times 10^6$/mL)	6.7 (4.9–9.6)	5.8 (4.6–8.6)	7.2 (5–10.1)	0.07
ANC ($\times 10^6$/mL)	5.2 (3–7.9)	4.3 (2.5–6)	5.7 (3.6–8.6)	**0.004***
ALC ($\times 10^6$/mL)	1.1 (0.8–1.5)	1.4 (1, 2)	1 (0.7–1.4)	**0.001***
AMC ($\times 10^6$/mL)	0.3 (0.2–0.5)	0.3 (0.2–0.5)	0.29 (0.16–0.44)	0.097
AEC ($\times 10^6$/mL)	0 (0–7.1)	5.3 (0–10.9)	0 (0–4.2)	**<0.001***
Platelets ($\times 10^6$/mL)	205 (154–269)	210 (165–240)	203.5 (153.3–282)	0.518
MCV (fL)	85 (83–89)	85 (82–89)	86 (83–89)	0.18
MCH (pg/cell)	29 (27–30)	29 (28–30)	29 (27–30)	0.54
MCHC (g/dL)	34 (33, 34)	34 (33–35)	33 (33, 34)	0.29
NLR	4.8 (2.2–9.1)	2.8 (1.6–4.6)	6.1 (2.7–11)	**<0.001***
PLR	192 (112–285)	129 (94–231)	213 (135–307.5)	**<0.001***
Ferritin (ng/mL)	350 (146.5–538.5)	171 (88–367)	440 (205–630)	**<0.001***

Abbreviations: ANC, absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; AEC, absolute eosinophil count; MCV mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; NLR, neutrophil/lymphocyte ratio; and PLR, platelet/lymphocyte ratio.

Based on the clinical report, 84 (50.9%) individuals were presented with at least one complication or co-morbidities in our study. Co-morbidities like hypertension (33.3%) and DM (15.8%) were predominant, followed by hypothyroidism, COPD, CVD, pulmonary disease, and others (Figure 1).

ANC (p = 0.004), NLR (p < 0.001), PLR (p < 0.001), and ferritin (p < 0.001) were significantly higher in severe cases in comparison to non-severe cases while ALC (p = 0.001), AEC (p < 0.001), and RBC (p = 0.037) were significantly lower in severe cases as compared to non-severe cases (Table 3). Similarly, positive CRP was significantly higher in severe cases than in non-severe cases (p < 0.001) (Figure 2). Furthermore, we observed that severe patients showed significantly increased glucose (p = 0.046), urea (p = 0.001), creatinine (p = 0.013), and AST (p = 0.030) levels than non-severe (Table 3 and Figure 3).

The crude odd ratio (Model I) of risk factors associated with COVID-19 infection was calculated using bivariate logistic regression analysis. Higher age, positive

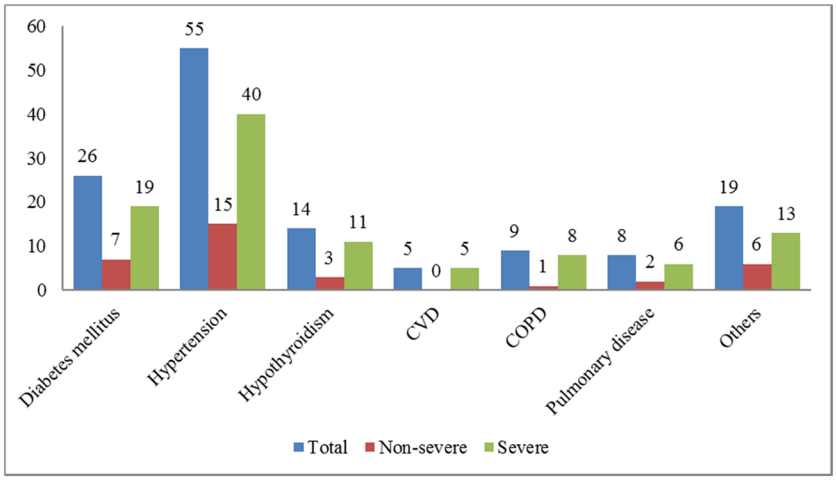

Figure 1: Distribution of co-morbidities among COVID-19 patients; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease.

Table 3: Biochemical findings of a patient with COVID-19 on admission to hospital

Biochemical parameters Median (IQR)	Total $N = 165$	Disease severity		*p*-Value
		Non-severe $N = 51$	Severe $N = 114$	
Glucose (g/dL)	136 (115–185)	133 (106–180)	141 (120–192)	**0.046***
Urea (mg/dL)	27 (18–39.5)	21 (15–33)	29.5 (20–50.25)	**0.001***
Creatinine (mg/dL)	0.8 (0.7–0.95)	0.8 (0.6–0.8)	0.8 (0.7–1)	**0.013***
Sodium (mEq/L)	136 (133–138)	135 (133–138)	136 (133–138)	0.442
Potassium (mEq/L)	4 (3.7–4.35)	4 (3.7–4.4)	4 (3.7–4.3)	0.660
Total bilirubin (mg/dL)	0.8 (0.7–0.9)	0.8 (0.7–0.8)	0.8 (0.7–0.9)	0.139
Direct bilirubin (mg/dL)	0.2 (0.2–0.2)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.163
ALP (U/L)	95 (67–118)	94 (67–118)	98 (67–118)	0.764
ALT (U/L)	45 (15, 28–58, 60–75)	40 (23.5–69)	47 (15, 31–58, 60–78)	0.066
AST (U/L)	53 (32–73.5)	40 (15, 23–58, 60–69)	54.5 (34–81.25)	**0.030***
AST/ALT	1.09 (0.84–1.46)	1.07 (0.82–1.47)	1.11 (0.84–1.47)	0.615

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; and AST, aspartate aminotransferase.
The bold letter signifies the statistically significant.

CRP, high ferritin, low ALC, NLR, PLR, high urea, and AST were individual risks associated with severe COVID-19 infection. The significant results obtained from Model I were adjusted in Model II and Model III, and multivariate regression analysis [odds ratio (OR) (95% confidence interval (CI))] was performed that showed higher age [3.611 (1.641–7.947)], positive CRP [2.930 (1.256–6.837)], high ferritin [2.754 (1.184–6.408)], low AEC [3.415 (1.544–7.552)] were independent risk factors associated with severe COVID-19 infection (Tables 4 and 5).

We tested the potential of significant prognostic markers (Age, NLR, PLR, Ferritin, and AEC) using AUROC curve analysis. The highest area under curve (AUC) (0.735) of ferritin was observed with an optimal cut-off value of 241 ng/mL, sensitivity and specificity of 74.0 and 65.0%, respectively, while the lowest AUC (0.661) of AEC with a cut-off of 35.5 cells/mm^3, sensitivity 74.0%, and specificity 67%. Similarly, at the cut-off of 47.5 years, age had AUC, sensitivity, and specificity of 0.724, 70.2, and 64.7%, respectively. NLR had AUC, cut-off, sensitivity, and specificity of 0.710, 3.3, 68.4, and 63.7%, respectively. Finally, the sensitivity and specificity of PLR were 77.2 and 51% at cut-off 129 with 0.678 AUC.

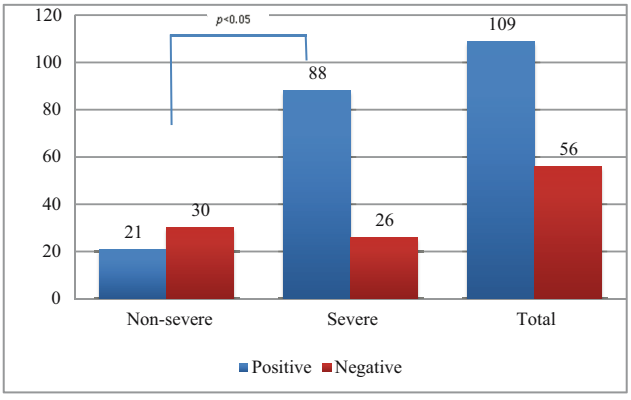

Figure 2: Distribution of CRP among COVID-19 patients.

4 Discussion

Prognostic markers used to measure the disease severity are considered useful in the patients' stratification into severe and non-severe groups. However, although most cases are mild to moderate with a better prognosis, the mortality rate was markedly higher in patients developing into severe cases. Therefore, the early identification of critical cases must reduce the mortality rate and improve the recovery rate by the early appropriate clinical intervention [29].

In our study, the mean ages of the study patients were 53.3 ± 16.5 years, which is similar to the study conducted by Chen et al. (55.5 years) [30], while older than that reported by Huang et al. (49 years) [29]. Most of the severe patients were older, with a mean age of 57.5 years than non-severe patients with a mean age of 44.4 years, which is in accordance with a study that suggested age may be a risk factor for poor outcome [30]. Moreover, the ROC curve for age was administered in our study, and the best cut-off point of age was 47.5 years which is similar to that of Yang et al. (49.5 years) [15], while younger than that of Wang et al. (52 years) [4]. Increasing age was

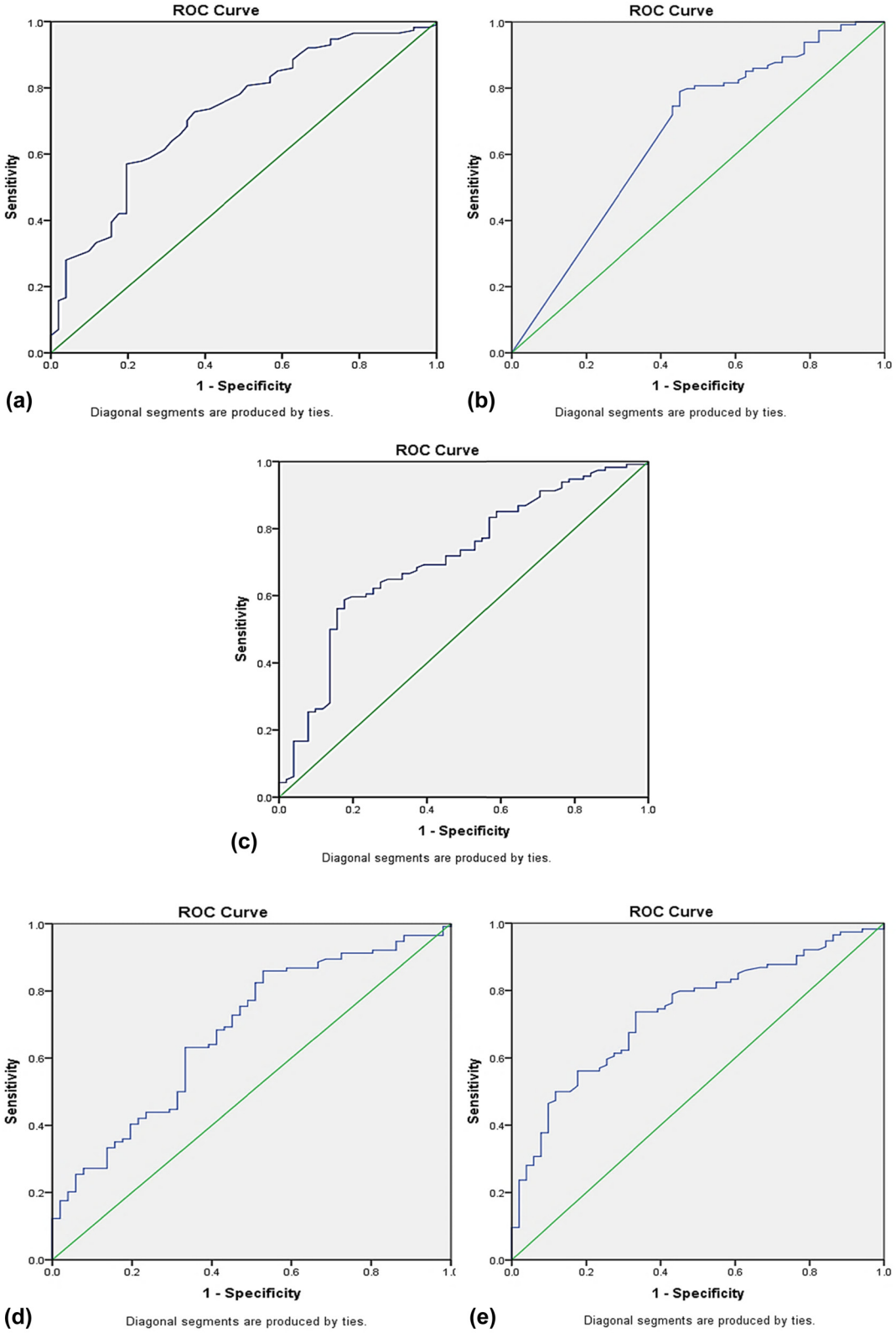

Figure 3: ROC curves of indicators on basis of COVID-19 severity: (a) age, (b) AEC, (c) NLR, (d) PLR, (e) ferritin.

Table 4: Logistic regression analysis of variables associated with severity of COVID-19

Test parameter	Model I	*p*-Value	Model II	*p*-Value	Model III	*p*-Value
Age (>53 years)	3.818 (1.876–7.771)	**0.001***	3.202 (1.364–7.516)	**0.008***	(1.641–7.947)	**0.001***
Gender (Male)	1.051 (0.491–2.248)	0.898	—	—	—	—
Co-morbidities	1.116 (0.576–2.160)	0.745	—	—	—	—
ANC (High)	2.245 (0.913–5.521)	0.078	—	-	—	-
ALC (Low)	2.824 (1.341–5.944)	**0.006***	1.173 (0.380–3.457)	0.773	—	—
AEC (Low)	3.568 (1.782–7.144)	**0.001***	2.702 (1.122–6.510)	**0.027***	3.415 (1.544–7.552)	**0.002***
NLR (>3.3)	3.649 (1.828–7.286)	**0.001***	0.774 (0.253–2.366)	0.654	—	—
PLR (>129)	3.351 (1.607–6.738)	**0.001***	1.749 (0.541–4.040)	0.445	—	—
Ferritin (High)	4.340 (2.516–8.738)	**0.001***	2.508 (0.980–6.420)	**0.049***	2.754 (1.184–6.408)	**0.019***
CRP (Positive)	4.835 (2.380–9.823)	**0.001***	2.810 (1.173–6.734)	**0.021***	2.930 (1.256–6.837)	**0.023***
Glucose (High)	1.833 (0.927–3.625)	0.081	—	—	—	—
Urea (High)	9.151 (2.098–39.912)	**0.003***	4.897 (0.898–26.704)	0.066	—	—
AST (High)	2.092 (1.059–4.131)	**0.034***	1.510 (0.642–3.554)	0.345	—	—

Odds ratios were given at 95% CI, and $p < 0.05$ was considered statistically significant.
The bold letter signifies the statistically significant.

found to be an independent risk factor for COVID-19 severity. Similar to our study, in a retrospective analysis of COVID-19 patients, multivariable regression showed an association of disease severity with older age (OR 1.10, 95% CI 1.03–1.17; $p = 0.0043$) [31]. In general, aged people are more prone to severity than younger people, which may be due to more health issues and underlying diseases in this population [32].

RBC was significantly associated with disease severity which may be due to pro-inflammatory cytokines released during COVID-19 infection resulting in blunt erythropoiesis [33]. However, in contrast to our study, other studies showed that RBC was not affected in COVID-19 patients [5,29,34,35].

In a study by Khalid and Ali Jaffar, leukocytosis was significant among COVID-19 patients [36]. Furthermore, a study of 140 hospitalized patients in Wuhan showed significantly higher leucocyte counts in severe cases than in milder forms [37]. The observed leukocytosis is contributed by the elevation of neutrophils as other WBC populations seem to decrease in severe cases of COVID-19 [38]. Similarly, our study demonstrated more neutrophils and fewer lymphocytes in severe cases compared to those in

Table 5: ROC curve for the severity of COVID-19

Test parameters	AUC	95% CI	*p*-Value	Cut-off value	Sensitivity%	Specificity%
Age (Years)	0.724	(0.641–0.807)	**0.001***	47.5	70.2	64.7
AEC (cells/mm^3)	0.661	(0.567–0.755)	**0.001***	35.5	74	67
NLR	0.710	(0.625–0.795)	**0.001***	3.3	68.4	63.7
PLR	0.678	(0.590–0.767)	**0.001***	129	77.2	51
Ferritin (ng/mL)	0.735	(0.656–0.813)	**0.001***	241	74	65

The bold letter signifies the statistically significant.

non-severe patients, similar to Xu et al. [5]. In addition, ANC was significantly higher in severe cases than in non-severe cases in our study, which corresponds well with other studies [29,32,37,39].

Absolute lymphocytopenia is primarily seen in COVID-19 patients, but significant lymphocytopenia is a cardinal marker of enhanced disease severity and indicator of mortality, which has been consistently depicted by several published reports and supported by our study [12,29,37,38]. During hospitalization, non-survivors established more advanced lymphocytopenia than recovered patients [32]. The significance of lymphocytopenia as a hematological symptom of COVID-19 infection has become evident from several studies, including that of Yang et al. showing 72.3% with lymphocytopenia [15], which is in concordance with our study (52.7%). Similarly, ALC was significantly lower in severe groups than in non-severe groups, supported by different studies [12,36]. Reasons for lymphocytopenia with disease severity may be due to the intensification of the inflammatory process (cytokine storm syndrome), direct infections of lymphocytes, and destruction of lymphoid organs, which is supported by the infection of T cells through receptor-dependent S protein-mediated membrane fusion which results in depletion of the cytotoxic capacity of lymphocytes [40–42].

Accordingly, eosinopenia has been reported in more than half (52.9–78.8%) of COVID-19 patients [37,39], which is in correspondence with our findings (65.5%). Similarly, AEC decreased significantly in severe as compared to that in non-severe cases, which is supported by Cai et al. [12]. Possible reasons for eosinopenia include viral attacking bone marrow and blocking eosinophil entrance to peripheral circulation [43]. In addition, AUC was found to be 0.717, which is similar to those of Li et al. (0.717) [43] and Seyit et al. (0.696) [44]. Similarly, our study supported the OR for eosinophil was found to be 3.41, which is supported by Li et al. (3.51) [43]. So, eosinophil, a routine parameter with other parameters, could be used to identify the highly suspected cases from mixed patients.

According to Liu et al., an increase in neutrophil count indicates the intensity of inflammatory response while lymphopenia suggests immune system disruption; therefore, high NLR may be a potential marker for risk factors [45]. NLR has also been a novel marker in other conditions, including thyroid nodules in patients with increased NLR in the preoperative period in case of underlying malignant nodular disease [46]. Our study depicted increased NLR among severe cases compared to not severe cases, similar to other studies [36,47,48]. Recent publications have suggested a high prognostic value of NLR for the prediction of disease severity with AUC 0.94 [12], 0.615 [44], and 0.689 [49], which supported our study that NLR was statistically significant between severe and non-severe groups with 0.710 AUC. Our cut-off value of NLR (3.3) was similar to Yang et al. [15], which showed a superior prognostic possibility of clinical symptoms to convert from mild to severe and similar to that of Liu et al., which gives 3.13. As per the regression analysis in our study performed, OR for NLR was 3.649, which was higher than that of Zhu et al. (1.090) [49] and Seyit et al. (1.274) [44]. Chinese retrospective study reported NLR, along with SARS-CoV-2 IgG used as a simple tool for the severity of COVID-19 infection and to predict clinical outcomes [29].

Increased PLR in COVID-19 patients is due to increased platelet activation and relative lymphocytopenia due to apoptosis of lymphocytes. The high PLR result in our study is consistent with a study [36]. The high PLR rate among COVID patients based on the length of hospital stay is also associated with the prognosis of the disease, which is also depicted by our study and several other studies [15,37,41,50]. Inflammation plays a key role in COVID-19 pathophysiology, with cytokine storm as a hallmark condition in severe disease and poor prognosis, whereas PLR is an established inflammatory marker [51,52]. Similarly, the PLR ratio was found to be 129 in our study, which is greater than that of the study performed by Seyit et al. [44], with a cut-off of 102.8, sensitivity of 70%, and specificity of 52%. In addition, we showed PLR as a risk factor for disease severity with COR 3.351, which is supported by a study with AOR 1.009 [44].

In our study, inflammatory markers, including ferritin and CRP, show a significant increase as indicated by Khalid and Ali Jaffar suggesting its association with disease severity and poor outcome, which may be associated with elevated hepcidin levels due to inflammatory reaction [36]. As per Kell and Pretorius ferritin levels greater than 600 ng/dL indicate cellular damage [53]. In our study, AUC for ferritin was found to be 0.735 with a cut-off of 241 ng/mL and an independent risk factor for the disease severity with AOR 2.754, which is supported by other findings [54]. It may be due to the extreme immune activation of cytokine storms in severe patients, leading to upregulation of serum ferritin levels [55]. CRP is an acute phase protein synthesized by hepatocytes, with increased levels parallel to the severity of inflammation [43]. In our study, 66.1% showed positive CRP with a higher population among severe cases (77.2%) than among non-severe cases (41.2%) in response to proinflammatory cytokines. According to Tan et al., CRP significantly increases among severe cases and predicts early

severe cases [56]. Our study found positive CRP as an independent risk factor for disease severity with OR 2.930.

In this study, biochemical parameters such as glucose, urea, creatinine, and AST had significant differences between severe and non-severe cases. Glucose is significantly higher in severe cases than in non-severe cases, similar to that of Gao et al. [57]. Creatinine was a significantly higher concentration in severe cases, supported by a study by Huang et al. [29]. In addition, 55.7 and 64.8% of patients showed elevated ALT and AST, respectively, as reported by Guan et al., which might show virus-mediated liver impairment. According to Zhang et al., any immune-mediated inflammation in particular cytokine storm and pneumonia-associated hypoxia may lead to liver damage in severe COVID-19 patients [58].

With the evaluation of the trends and variations of CBC and inflammatory parameters among severe and non-severe COVID-19 patients, it can be concluded that the use of readily available, inexpensive tests could be helpful for the diagnosis, prognosis, stratification, and prioritization of treatment in the unavailability of the standard test like RT-PCR, and CT- scan in the developing countries lacking the infrastructures and manpower with a heavy burden of the diseases.

This study has several limitations. First, some inflammatory factors and immunological indexes cannot be detected and compared due to the limitation of experimental conditions. Second, this is a cross-sectional study with participants from a single center rather than multiple centers.

5 Conclusion

In comparison to non-severe COVID-19, severe COVID-19 was associated with increased markers of the innate immune response such as neutrophil count, NLR, CRP, and serum ferritin; decreased markers of the adaptive immune response such as lymphocyte, and increased markers of significant organ damage including AST, urea, and creatinine. Moreover, age, AEC, CRP, and ferritin were independent risk factors for assessing the severity of COVID-19. Therefore, with the following parameters such as age > 47.5 years, NLR > 3.3, PLR > 129, ferritin > 241 ng/mL, and AEC < 35.5 cells/cumm, the progress of COVID-19 to the severe stage should be closely observed and managed accordingly.

Additionally, hematological and inflammatory parameters can aid clinicians in determining the severity of disease that can help prioritize cases and provide prompt treatment reducing the mortality rate.

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