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Comorbidities influence the predictive power of hematological markers for mortality in hospitalized COVID-19 patients

Abstract

Introduction: Coronavirus disease 2019 (COVID-19) pandemic has caused unprecedented mortality and has stretched the health infrastructure thin worldwide, especially in low- and middle-income countries. There is a need to evaluate easily available biomarkers for their clinical relevance for poor outcomes in severe cases of COVID-19. It is also known that comorbidities affect these biomarkers with or without COVID-19. We aimed to unearth the influence of comorbidities on feasible hematological predictive markers for mortality in hospitalized severe COVID-19 patients.

Materials and methods: This is a retrospective study done on severe COVID-19 hospitalized patients, diagnosed with RT (real time) polymerase chain reaction ($n = 205$), were investigated. Comorbidities associated with the patients were tracked and scored according to Charlson comorbidity index (CCI). CCI score of zero was grouped in A, those with CCI score 1–4 into group B and those with CCI scores ≥ 5 into group C. Correlation between hematological parameters and CCI scores was analyzed using Spearman correlation coefficient. Optimal cut-off and odds ratio was derived from receiver operating characteristic (ROC) curve analysis.

Results: Among the 205 severe COVID-19 patients age, C-reactive protein (CRP), neutrophil lymphocyte ratio (NLR), derived NLR (dNLR), absolute neutrophil count (ANC) and total leukocyte count (TLC) were found to be statistically significant independent risk factors for predicting COVID-19 mortality ($p < 0.01$). In group A, cut off for CRP was 51.5 mg/L (odds ratio [OR]: 26.7; area under curve [AUC]: 0.867), TLC was 11850 cells/mm^3 (OR: 11.7; AUC: 0.731), NLR was 11.76 (OR: 14.3; AUC: 0.756), dNLR was 5.77 (OR: 4.89; AUC: 0.659), ANC was 13110 cells/mm^3 (OR: 1.68; AUC: 0.553). In group B, cut off for CRP was 36.5 mg/L (OR: 32.1; AUC: 0.886), TLC was 11077 cells/mm^3 (OR: 12.1; AUC: 0.722), NLR was 8.27 (OR: 18.9; AUC: 0.827), dNLR was 3.79 (OR: 9.26; AUC: 0.727), ANC was 11420 cells/mm^3 (OR: 2.42; AUC: 0.564). In group C, cut-off for CRP was 23.7 mg/L (OR: 32.7; AUC: 0.904), TLC was 10480 cells/mm^3 (OR: 21.2; AUC: 0.651), NLR was 6.29 (OR: 23.5; AUC: 0.647), dNLR was 1.93 (OR: 20.8; AUC: 0.698), ANC was 6650 cells/mm^3 (OR: 2.45; AUC: 0.564).

Conclusions: In severe COVID-19 patients, CRP was the most reliable biomarker to predict mortality followed by NLR. Presence, type, and number of co-morbidities influence the levels of the biomarkers and the clinically relevant cut-offs associated with mortality.

Key words: COVID-19, SARS-CoV 2, comorbidities, prognostic indicators, hematological markers, neutrophil lymphocyte ratio, C-reactive protein

Adv Respir Med. 2022; 90: 49–59

Introduction

The coronavirus disease (COVID) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019 (Wuhan city, China) [1]. The first COVID-19 case was reported in India in late January 2020, and in March, the World Health Organization officially classified the disease outbreak as a pandemic [2]. The infection spread like wildfire, and by May of 2021, it is responsible for 165 million cases and 3.4 million deaths world-

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DOI: 10.5603/ARM.a2022.0017 | Received: 23.05.2021 | Copyright © 2022 PTChP | ISSN 2451–4934 | e-ISSN 2543–6031

wide [3]. Healthcare resource facilities around the world have been stretched thin and experienced widespread shortages. This is especially true in low- and middle-income countries (LMIC) centers [4]. Advanced biomarker testing may not be available in many LMIC centers and there is a need to identify simple biomarkers of mortality to plan early interventions.

Hematological parameters, being a simple investigation and widely available, is a lesser strain on medical and financial resources and have been observed to predict outcomes in COVID 19 patients [5–7]. Hemoglobin (Hb), platelet count, neutrophil-lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), red cell width are simple biomarkers and predict death in COVID-19 patients (7).

Pre-existent comorbidities such as diabetes, hypertension, chronic kidney disease worsen COVID-19 outcomes (8–10). However, to date, studies that have evaluated the role of immune-hematological markers in COVID-19 progression have not considered the confounding effects of pre-existent co-morbidities on the cut-offs associated with poor outcomes. This is extremely important as the predictive values and optimal thresholds of these markers may be different in the high-risk groups which have increased levels of immune-hematological markers even without COVID-19 infection [11–15].

Comorbidities can be defined as diseases that coexist with the disease of interest, which may directly affect the outcome of the disease of interest [16–18]. The most commonly widely accepted comorbidity scoring system is the Charlson comorbidity index (CCI) [16, 19]. It contains a total of 19 different comorbidities which are assigned a score weighted according to their possible impact on mortality. Many co-morbid conditions have been shown to influence the hematological markers even in the absence of COVID-19 [20–23]. No study has evaluated the impact of co-morbid conditions, whether suffering from multiple co-morbidities influences the optimal cut-offs in the receiver operating characteristic (ROC) analysis and whether multiple co-morbidities influence the odds of mortality for common hematological markers.

Therefore, the objective of the present study was to evaluate the influence of comorbidities on prognostic predictive potential for various immune-hematological markers for COVID-19 mortality in the hospitalized severe COVID-19 patients.

Materials and methods

Data collection

This is a retrospective study conducted in COVID-19 positive patients admitted at a tertiary care university teaching hospital at Mysuru, from July 6, 2020, to October 30, 2020. This study was approved by the Institutional Ethics Committee of JSS Medical College, Mysuru (Approval number: JSSMC/IEC/141020/09 NCT)/2020-21).

The demographic profile, clinical, hematological, treatment, and survival outcomes were collected from medical records in a predetermined data collection sheet by physicians and validated by another researcher. Hematological investigations and C-reactive protein (CRP) were performed at the time of admission and included baseline Hb%, PCV, total leucocyte count, neutrophil count, lymphocyte count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), platelet, red cell width (RDW) and CRP. Hematological investigations were carried out on automated blood analyzer Sysmex XN 1000. NLR, derived NLR (dNLR) and PLR were calculated by the formula 'NLR = absolute neutrophil count/absolute lymphocyte count', dNLR = absolute neutrophil count/(Total leukocyte count – absolute neutrophil count) and 'PLR = platelet count/absolute lymphocyte count'. Treatment for COVID-19 was given as per the protocol based on the Ministry of Health and Family Welfare guidelines, Government of India [24].

Chest X-rays of the patients which was done during initial presentation to the hospital was gathered and scored as proposed by Warren et al. [25]. Each lung was given a score of 0–4 depending on the lung involvement (score 0 = no involvement; 1 ≤ 25%; 2 = 25–50%; 3 = 50–75%; 4 ≥ 75% lung involved). The total severity score was calculated by adding both lung scores (ranging from 0 to 8).

Diagnostic criteria

The inclusion criteria for the study were patients aged more than 18 years, diagnosed with severe COVID-19 by SARS-CoV-2 RNA detection in throat swab specimens via polymerase chain reaction (as per NIH COVID-19 treatment guidelines [26]).

The severe group included COVID-19 patients with SpO_2 < 90% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mmHg, respiratory frequency > 30 breaths/min, or lung infiltrates > 50%, respiratory failure, septic shock, and/or multiple organ dysfunction.

Patients with hematological malignancies, immunodeficiency states, pregnant mothers, and children were excluded from the study. Furthermore, those with only OPD (Outpatient Department) visits, missing clinical and hematology data, discharged against medical advice, transfer to other medical facilities, were excluded from the study. Comorbidities were recorded for all the patients and were scored according to the CCI scoring system [19].

Statistical analysis

For the data of continuous variables, the Shapiro-Wilk test for normality was first conducted. It was found that the hematological parameters were not normally distributed. These parameters were represented as the median (interquartile range [IQR]). Statistical significance was assessed by Pearson's chi-square test for categorical variables and by Student's T or Kruskal–Wallis test for continuous variables depending on the distribution of data. Correlation analysis of CCI with hematological parameters was done using Spearman's correlation test due to the non-normal distribution of data. Both univariable and multivariable binomial logistic regression was performed to assess the variables independently associated with COVID-19 mortality.

Categorical variables were summarized as frequency and percentages in each category. Those with no comorbidities i.e., CCI score of zero were grouped in group A, those with CCI score 1–4 into group B and those with CCI scores ≥ 5 into group C. Evaluation of hematological parameters in predicting mortality due to COVID-19 was by calculating the sensitivity, specificity, the ROC and the area under the curve (AUC). Youden's index (sum of sensitivity and specificity minus one) was used to obtain optimal cut-off thresholds. All the statistical analyses were performed using Jamovi v1.16 (The Jamovi Project, Sydney, Australia) and SPSS-IBM software (SPSS Inc v20, Chicago, USA)

Results

A total of 205 cases were included in this study, 144 (70.2%) males and 61 (29.8%) females. The median age of the study population was 58 years. Patients in group C had the oldest age group followed by group B and A (65.8 vs. 57.7 vs. 43.5; $p < 0.01$). More than one-quarter (26.8%) succumbed to COVID-19 related death (Table 1). The most common comorbidity found among the study population was diabetes mellitus (47.8%), followed by hypertension (43.9%), while the least was autoimmune diseases (1%). Those with comorbidities were in a higher proportion in group C than group A and B. This was statistically significant i.e. $p < 0.05$ for all comorbidities (Table 2).

Most of the hematological parameters showed greater impairment in COVID-19 patients with group C than in those in group A or B. COVID-19 patients in group C had lower hemoglobin and platelet counts, higher TLC, ANC, NLR, dNLR, RDW, and CRP as compared to patients in group A or B (Table 1). Binomial logistic regression showed that age (adjusted odds ratio [AOR]: 1.03 [1.00––1.06], $p = 0.025$), CRP (AOR: 1.94 [1.03–3.67], $p = 0.042$), ANC (AOR: 1.06 [1.00–1.13], $p = 0.044$), NLR (AOR: 1.59 [1.23–2.64], $p = 0.010$), dNLR (AOR = 1.07 [1.04–1.10], $p < 0.001$), and TLC (AOR: 1.42 [1.08–1.88], $p = 0.013$) were independent risk factors for mortality in cases of COVID-19 (Table 3).

Using Spearman's correlation test we evaluated hematological parameters with the CCI scores. We observed that CCI scores were positively correlated with TLC, ANC, NLR, dNLR, and CRP (Table 4). The ROC were drawn separately of NLR, dNLR, TLC, ANC, and CRP in COVID-19 subjects for different groups. The AUC and optimal cut-off thresholds for each of the independent risk factors and predictive odds ratios for mortality are shown in Table 5 and Figure 2, respectively. The AUC was greater for CRP in comparison to NLR in predicting COVID-19 associated mortality in all the groups.

C-reactive protein

C-reactive protein, an inflammatory marker, was a statistically significant independent risk factor for COVID-19 related mortality and was influenced by the presence and number of co-morbidities. CRP in the patients with no comorbidities i.e., group A was 70.8 mg/L, group B was 82.1 mg/L and group C was 93.8 mg/L (Table 1).

A CRP cut-off of 51.5 mg/L had a sensitivity of 74.51% and specificity of 78.95% (using Youden's index), an AUC of 0.812, and the odds of 26.7 for COVID-19 related mortality in Group A patients. In the case of the patients in group B, the cut-off was slightly lower 36.5 mg/L with an AUC of 0.866 (sensitivity: 90.24% and specificity: 64.86%) and an odds of 32.1 to suffer from COVID-19 related mortality. The cut-off for group C was the lowest at 23.7 mg/L with an AUC of 0.903 (sensitivity: 80.33% and specificity: 96.00%) and the odds of 32.7 to suffer from COVID-19 related mortality (Figures 1 and 2)

Table 1. Demographic and hematological parameters of the study population

Parameters	CCI score groups			P-value
	Group A (n = 81)	Group B (n = 83)	Group C (n = 41)	
Age [years]	43.5 (27.7–59.3)	57.7 (44.9-70.5)	65.8 (53.5-78.1)	< 0.01*
Sex				
Female	22 (32.8%)	15 (25.4%)	23 (29.5%)	0.69#
Male	45 (67.2%)	44 (74.6%)	55 (70.5%)	
Death (n/total)	18/81	25/83	15/41	0.22#
CXR scores	3.11 (1.28–4.94)	3.42 (1.10–5.74)	3.60 (1.37–5.83)	0.42*
Hematological Investigations				
Hemoglobin percentage [mg/dL]	13 (11.06–14.94)	12.7 (9.85–15.55)	11.8 (9.19–14.41)	< 0.01*
Packed cell volume [%]	38.9 (33.8–44)	37.8 (30.09–45.51)	35.4 (27.9–42.9)	< 0.01*
Total leucocyte count in [cells/mm^3]	9970 (5885–14055)	10334 (5180–15488)	13303 (7895–18711)	0.006*
Absolute lymphocyte count [cells/mm^3]	1475 (493–2457)	1248 (461–1709)	1143 (608–1678)	< 0.01*
Platelets [$\times 10^5$ cells/mm^3]	2.82 (1.73–3.91)	2.67 (1.14–3.81)	2.52 (1.58–3.46)	0.34*
Absolute neutrophil count in [cells/mm^3]	7435 (2678–12192)	8096 (2625–13567)	11436 (5222–17650)	0.04*
Neutrophil lymphocyte ratio	7.49 (0.34–14.64)	9.22 (0.26–18.18)	14.6 (1.8–27.4)	< 0.01*
Derived neutrophil lymphocyte ratio	4.5 (1.19–7.81)	6.85 (2.58–11.12)	7.42 (4.25–10.59)	< 0.01*
Platelet lymphocyte ratio	240 (88–392)	289 (21–557)	331 (167–501)	0.19*
Red cell distribution width [%]	13.6 (12.47–14.73)	14.7 (12.81–16.59)	15 (13.58–16.42)	0.01*
C-reactive protein [mg/L]	70.8 (9.5–132.1)	82.1 (20.5–143.7)	93.8 (3.2–184.4)	0.01*

*Kruskal–Wallis test, #Pearson's chi^2. Hematological parameters are presented as the median values with interquartile range in the brackets. Charlson's comorbidity index (CCI) score of zero was grouped in A, those with CCI score 1–4 into group B and those with CCI scores ≥ 5 into group C

Table 2. Comorbidities in the study population

		Group A (n = 81)	Group B (n = 83)	Group C (n = 41)	Total (n = 205)	P-value
Diabetes mellitus	No	81 (100.0%)	19 (22.9%)	7 (17.1%)	107 (52.2%)	< 0.001*
	Yes	0 (0.0%)	64 (77.1%)	34 (82.9%)	98 (47.8%)	
Hypertension	No	81 (100.0%)	32 (38.1%)	2 (4.9%)	115 (56.1%)	< 0.001*
	Yes	0 (0.0%)	51 (61.9%)	39 (95.1%)	90 (43.9%)	
Chronic kidney disease	No	81 (100.0%)	83 (100.0%)	28 (68.3%)	192 (93.7%)	< 0.001*
	Yes	0 (0.0%)	0 (0.0%)	13 (31.7%)	13 (6.3%)	
Ischemic heart disease	No	81 (100.0%)	77 (92.8%)	18 (43.9%)	176 (85.9%)	< 0.001*
	Yes	0 (0.0%)	6 (7.2%)	23 (56.1%)	29 (14.1%)	
Chronic lung disease	No	81 (100.0%)	78 (94.0%)	35 (85.4%)	194 (94.6%)	0.003*
	Yes	0 (0.0%)	5 (6.0%)	6 (14.6%)	11 (5.4%)	
Others#	No	81 (100.0%)	80 (96.4%)	32 (78.0%)	193 (94.1%)	< 0.001*
	Yes	0 (0.0%)	3 (3.6%)	9 (22.0%)	12 (5.9%)	

*Pearson's chi^2. #Others: autoimmune diseases, HIV, chronic liver disease, cerebrovascular disease. Charlson's comorbidity index (CCI) score of zero was grouped in A, those with CCI score 1–4 into group B and those with CCI scores ≥ 5 into group C

Neutrophil-lymphocyte ratio

Neutrophil-lymphocyte ratio was found to be a statistically significant independent risk factor for COVID-19 related mortality. NLR in the patients with no comorbidities i.e., group A was 7.49, group B was 9.22, and group C was 14.6 (Table 1).

In group A, we derived sensitivity of 60% and specificity of 87.04% (using Youden's index)

Table 3. Binomial logistic regression analysis of variables associated with COVID-19 mortality

Variables	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age [years]	1.04 (1.02–1.06)	0.001	1.03 (1.00–1.06)	0.025
Sex (ref: males)	0.97 (0.49–1.87)	0.930	0.93 (0.45–1.86)	0.841
Chest X-ray scoring	1.13 (0.94–1.85)	0.571	1.06 (0.93–1.71)	0.616
Comorbidities				
Diabetes mellitus	1.03 (0.56–1.89)	0.932	0.63 (0.31–1.28)	0.205
Hypertension	1.71 (0.93–3.18)	0.085	1.22 (0.58–2.54)	0.601
Chronic kidney disease	1.14 (0.30–3.65)	0.838	1.00 (0.24–3.54)	0.996
Ischemic heart disease	1.68 (0.72–3.77)	0.217	0.86 (0.32–2.20)	0.754
Chronic lung disease	1.48 (0.38–5.11)	0.543	0.88 (0.21–3.24)	0.849
Others	1.29 (0.33–4.26)	0.690	1.11 (0.27–3.94)	0.875
Hematological investigations				
Hemoglobin percentage [mg/dL]	0.86 (0.74–0.99)	0.034	1.63 (0.73–3.83)	0.243
Packed cell volume [%]	0.94 (0.90–0.99)	0.020	0.85 (0.64–1.13)	0.279
Total leucocyte count in [cells/mm^3]	1.12 (1.05–1.20)	< 0.001	1.42 (1.08–1.88)	0.014
Absolute neutrophil count in [cells/mm^3]	1.11 (1.06–1.17)	< 0.001	1.06 (1.00–1.13)	0.044
Absolute lymphocyte count [cells/mm^3]	1.86 (1.13–3.06)	0.014	1.28 (0.75–2.18)	0.369
Platelets [$\times 10^5$ cells/mm^3]	1.20 (0.84–1.69)	0.309	1.28 (0.74–2.17)	0.367
Neutrophil lymphocyte ratio	1.40 (1.23–1.68)	< 0.001	1.59 (1.23–2.64)	0.010
Derived neutrophil lymphocyte ratio	1.09 (1.06–1.11)	< 0.001	1.07 (1.04–1.10)	< 0.001
Platelet lymphocyte ratio	1.00 (1.00–1.00)	0.665	1.00 (1.00–1.00)	0.581
Red cell distribution width [%]	1.06 (0.99–1.26)	0.332	1.05 (0.99–1.26)	0.258
C-reactive protein [mg/L]	1.09 (1.04–1.15)	< 0.001	1.94 (1.03–3.67)	0.042

CI — confidence interval; OR — odds ratio

with an AUC of 0.756. An applicable cut-off of 11.76 was established with an odds ratio of 14.3 for COVID-19 related mortality. The cut-off for group B was lower at 8.27 with an AUC of 0.845 (sensitivity: 66.67% and specificity: 92.00%) and the odds of 18.9 to suffer from COVID-19 related mortality. In the case of patient group C, the cut-off was the lowest at 6.29 with an AUC of 0.647 (sensitivity: 65.22% and specificity: 61.22%) and the odds of 23.5 to predict mortality (Figures 1D and 2)

Derived neutrophil-lymphocyte ratio

Derived NLR was found to be a statistically significant risk factor for COVID-19 related mortality. dNLR in the patients with no comorbidities i.e., group A was 4.5, group B was 6.85, and group C was 7.42 (Table 1).

A dNLR with a cut-off of 5.77 had a sensitivity of 56.60% and specificity of 78.95% (using Youden's index), an AUC of 0.659, and the odds of 4.89 to suffer from COVID-19 related mortality in group A patients. In the case of the patient group B, the cut-off was slightly lower at 3.79 with an AUC of 0.727 (sensitivity: 71.88% and specificity: 71.88%) and an odd of 9.26 to suffer from COVID-19 related mortality. The cut-off for group C was the lowest at 1.93 with an AUC of 0.698 (sensitivity: 97.37% and specificity: 36%) and the odds of 20.8 to suffer from COVID-19 related mortality (Figure 2).

Total leucocyte count

Total leucocyte count was found to be a statistically significant independent risk factor for COVID-19 related mortality. TLC in the patients with no comorbidities i.e., group A was 9970 cells/mm^3, group B was 10334 cells/mm^3 and group C was 13303 cells/mm^3 (Table 1).

For those without comorbidities i.e., group A, we derived sensitivity of 60% and specificity of 83.33% (using Youden's index) in the

Table 4. Correlation between Charlson's comorbidity index (CCI) scores and hematological parameters

	CCI scores	
	Spearman's rho	P-value
C-reactive protein	0.294	< 0.001
Neutrophil-lymphocyte ratio	0.253	< 0.001
Derived neutrophil-lymphocyte ratio	0.203	0.004
Absolute neutrophil ratio	0.158	0.023
Total leukocyte ratio	0.154	0.027

ROC curve, with an AUC of 0.731. An applicable cut-off of 11850 cells/mm^3 was established with an odds ratio of 11.7 to predict suffering COVID-19 related mortality in patients with comorbidities. In the case of patient group B, the cut-off was 11077 cells/mm^3 with an AUC of 0.722 (sensitivity:50% and specificity: 86.36%), and the odds of predicting suffering COVID-19 related mortality was 12.1. The cut-off for group C was the lowest at 10480 cells/mm^3 with an AUC of 0.651 (sensitivity: 65.22% and specificity: 67.35%) and the odds of 21.2 to suffer from COVID-19 related mortality (Figure 2).

Absolute neutrophil count

Absolute neutrophil count was found to be a statistically significant independent risk factor for COVID-19 related mortality. ANC in the patients with no comorbidities i.e., group A was 7435 cells/mm^3, group B was 8096 cells/mm^3 and group C was 11436 cells/mm^3 (Table 1).

For those without comorbidities i.e., group A, we derived sensitivity of 44.44% and specificity of 63.33% (using Youden's index) in the ROC curve, with an AUC of 0.553. An applicable cut-off of 13110 cells/mm^3 was established with an odds ratio of 1.68 to predict suffering COVID-19 related mortality in patients with comorbidities. In the case of patient group B, the cut-off was lower at 11420 cells/mm^3 with an AUC of 0.564 (sensitivity: 31.58% and specificity: 84%) and the odds of predicting suffering COVID-19 related mortality was 2.42. The cut-off for group C was the lowest at 6650 with an AUC of 0.564 (sensitivity: 65.79%and specificity: 56%) and the odds of 2.45 to suffer from COVID-19 related mortality (Figure 2).

Discussion

We observed that CRP, NLR, dNLR, TLC, and ANC had a varying degree of accuracy in the abil-

Table 5. Area under the curve (AUC) with sensitivity, specificity, and AUC for mortality related to COVID-19 derived from the receiver operating characteristics curve for those with different Charlson comorbidity index scores

Variable	Comorbidities	Sensitivity (%)	Specificity (%)	AUC
Total leukocyte ratio	Group A	60.00%	83.33%	0.731
	Group B	50.00%	86.36%	0.722
	Group C	65.22%	67.35%	0.651
Absolute neutrophil ratio	Group A	44.44%	63.33%	0.553
	Group B	31.58%	84.00%	0.564
	Group C	65.79%	56.00%	0.564
Neutrophil-lymphocyte ratio	Group A	60.00%	87.04%	0.756
	Group B	66.67%	92.00%	0.845
	Group C	65.22%	61.22%	0.647
Derived neutrophil-lymphocyte ratio	Group A	56.60%	78.95%	0.659
	Group B	71.88%	71.88%	0.727
	Group C	97.37%	36.00%	0.698
C-reactive protein	Group A	74.51%	78.95%	0.812
	Group B	90.24%	64.86%	0.866
	Group C	80.33%	96.00%	0.903

Charlson's comorbidity index (CCI) score of zero was grouped in A, those with CCI score 1–4 into group B and those with CCI scores ≥ 5 into group C

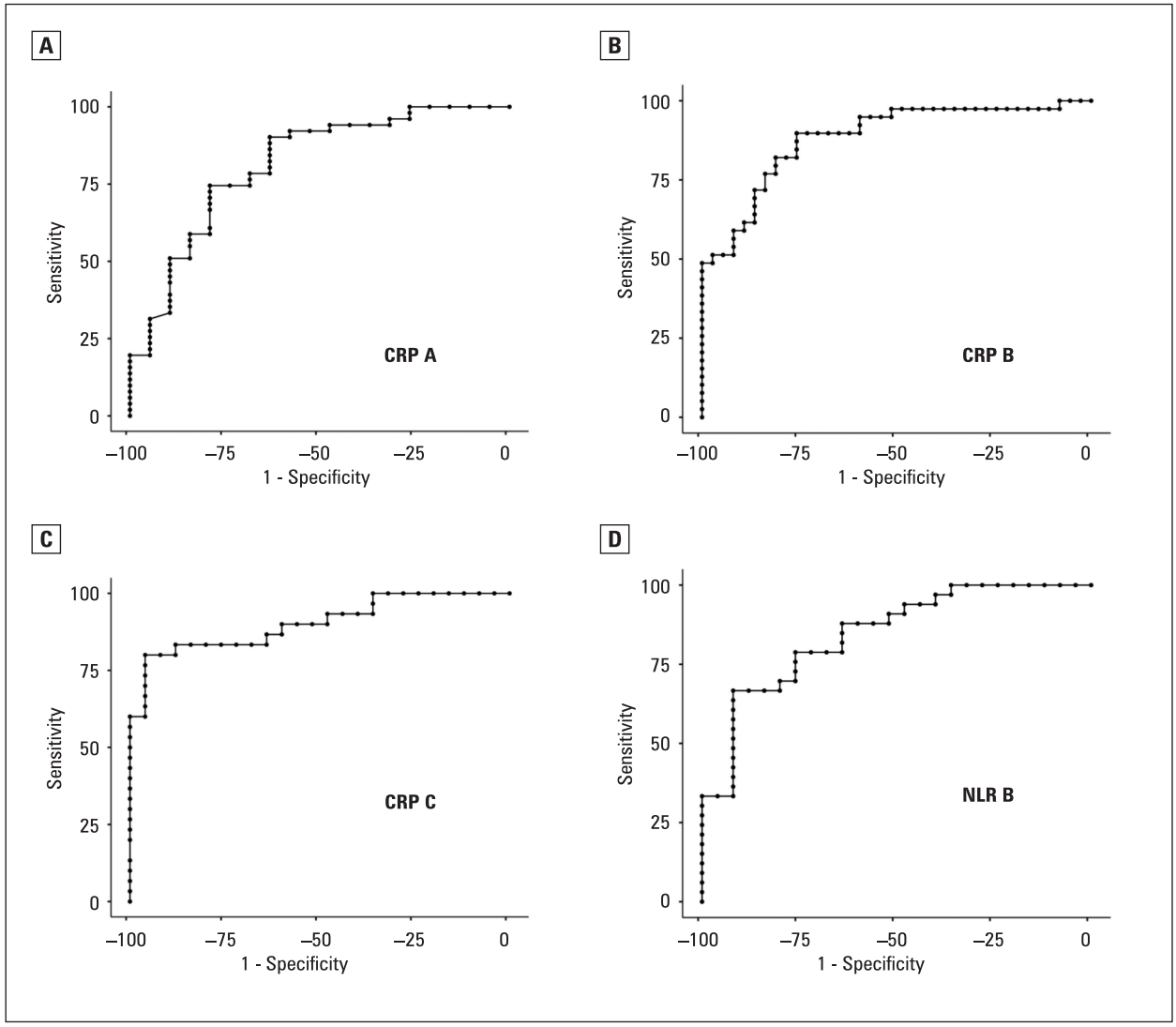

Figure 1. A. The figure illustrates receiver operating characteristic curve (ROC) analysis for C-reactive protein (CRP) for group A. The groups are based on Charlson's comorbidity index (CCI) scores and a CCI score of zero was grouped in A; **B.** The figure illustrates ROC analysis for CRP for group B. The groups are based on CCI scores and those with CCI scores of 1–4 were grouped into Group B; **C.** The figure illustrates ROC analysis for CRP for Group C. The groups are based on CCI scores and those with CCI scores ≥ 5 into group C; **D.** The figure illustrates ROC analysis for neutrophile-lymphocyte ratio (NLR) for group B. The groups are based on CCI scores and those with CCI score of 1–4 was grouped into group B

ity to predict mortality in cases of COVID-19 with and without comorbidities. Other markers such as platelet counts, PLR, RDW, hemoglobin were not useful to predict risk for mortality. The most important observation from our study that has not been discussed in previous studies is that the values of these hematological inflammatory biomarkers are dependent on the presence of co-morbidities and multiple co-morbidities have a greater impact. We observed that as the number of comorbidities increases, the mean values of the biomarkers in patients increase. The cut-off of the biomarkers associated with increased risk of mortality is lower in patients with comorbidities compared to patients with COVID-19 without any co-morbidities. Even with lower cut-offs, the odds of mortality were higher in the patients with comorbidities when compared to those without comorbidities.

Many co-morbidities such as diabetes, hypertension, chronic kidney disease, and various carcinomas would lead to elevated levels of inflammatory markers even without COVID-19 infections [11, 13–15, 27–29]. We explored whether these hematological markers would be different in COVID-19 subjects with co-morbidities in comparison to subjects without co-morbidities and observed that co-morbidities such as diabetes, hypertension, ischemic heart disease, and chronic kidney disease influence the levels of these in-

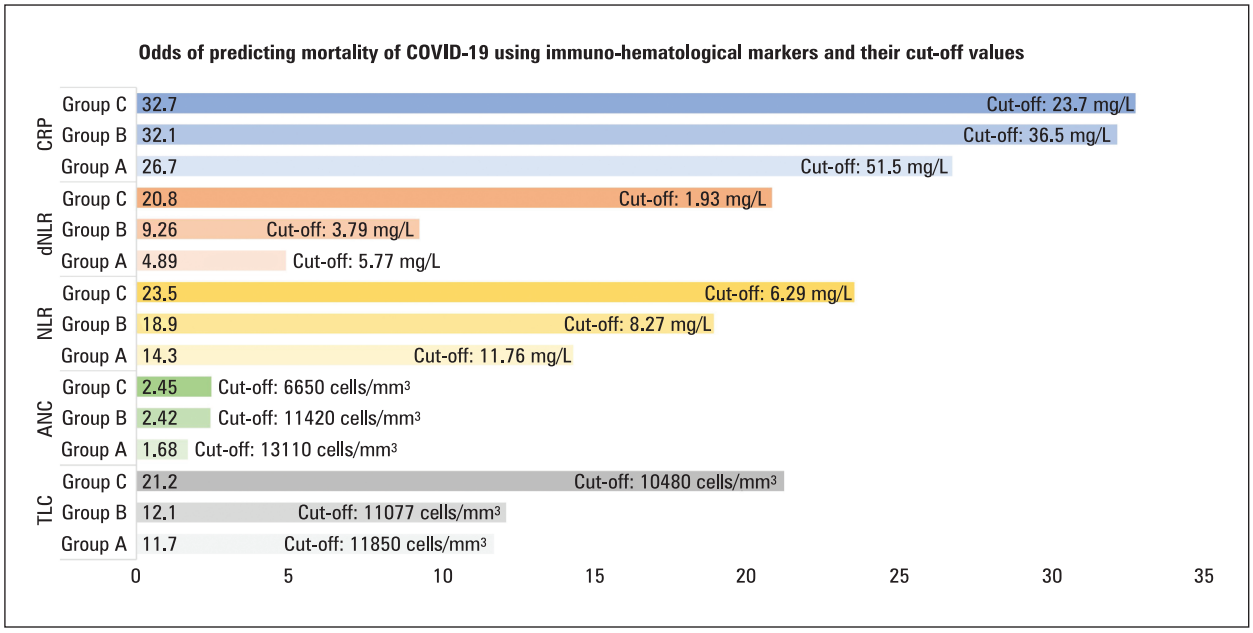

Figure 2. The figure illustrates the odds of predicting mortality using immuno-hematological markers and their optimal cut-off values. The groups are based on Charlson's comorbidity index (CCI) scores. The cut-off of the biomarkers associated with mortality is lower in patients with comorbidities (group B and C). Even with lower cut-offs, the odds of mortality were higher in the patients with comorbidities (group B and C) when compared to those without comorbidities (group A). CCI score of zero was grouped in A, those with CCI score 1–4 into group B and those with CCI scores ≥ 5 into group C

flammatory markers in COVID-19 patients. Therefore, it is necessary to identify different cut-offs to predict mortality in COVID-19 subjects with and without co-morbidities.

There is an abundant synthesis of acute-phase proteins predominantly CRP by hepatocytes as a response to infection [30, 31]. CRP, being a non-specific acute-phase proteins, is secreted as early as 4 hours after an inflammatory insult and peaks at 48 hours. It has a short half-life of 19 hours [32]. The most important property of CRP is that it may be raised before the raise of leukocytes or altered vital signs [32]. This makes CRP as a biomarker a useful tool for diagnostics. There is evidence of a correlation between elevated CRP levels and COVID-19 disease progression [33]. Furthermore, a systematic review observed that a significant increase in CRP in 73% of the COVID-19 patients and was the most prevalent impaired laboratory finding [34]. In our study, CRP was found to have the highest probability of predicting mortality related to COVID-19 in patients from all three groups (CCI score 0, CCI score 1–4, and CCI > 5). Similarly, the AUC for CRP was the highest for COVID 19 mortality among all biomarkers in subjects with and without co-morbidities.

COVID-19 spreads rapidly and is classified as a global pandemic affecting high-income countries and LMIC alike. Researchers have been making efforts to unearth not only the most effective but also an economical prognostic tool for COVID-19 mortality. Hematological markers such as lymphopenia, leukocytosis with an increased neutrophil count, and thrombocytopenia, were found to positively correlate with disease progression as well as being most economical [35–37].

Neutrophile to lymphocyte ratio (NLR = ANC/absolute lymphocyte counts) was first proposed to be used as a prognostic marker in patients who are critically ill as it correlated well with APACHE II and SOFA scores [38]. It has also been used in the diagnosis of bacteremia, influenza virus infection, and Middle East respiratory syndrome (MERS) [39, 40]. A systematic review of several studies for COVID-19 assessed that NLR is a good prognostic tool for mortality (AUC: 0.90) with a cut-off ranging from 3.0 to 13.4 in different studies [41]. NLR was found to be a strong predictive tool in our study as well, with an AUC: 0.756 (cut-off: 11.76) for mortality in patients with no comorbidities. NLR also had a robust capacity to predict mortality in patients with a CCI score between 1 and 4 (AUC: 0.827, cut off: 8.27) and patients with a CCI score > 5 (AUC: 0.647, cut off: 6.29). Derived NLR is a prognostic immuno-biomarker, commonly used in oncology [27, 29, 42]. Derived NLR, as a prognostic tool

for COVID-19, is a new marker that has not been assessed as extensively as NLR. We observed that it is inferior to NLR to predict the risk of mortality in our study subjects.

In response to the COVID-19 infection, we see a rise in the leukocytes (predominantly neutrophils) that increase the TLC [43, 44], which in our study showed a significant predictive power in predicting mortality in patients without comorbidities (AUC: 0.731, cut-off: 11850 cells/mm^3). TLC also had a robust capacity to predict mortality in patients with a CCI score between 1 and 4 (AUC: 0.722, cut-off: 11077 cells/mm^3) and patients with a CCI score > 5 (AUC: 0.651, cut-off: 10480 cells/mm^3).

These findings are of great value as NLR, dNLR, and TLC are a simple cost-effective predictive tool available, affordable to residents even in remote areas with access to only primary care, which offers an enormous economic advantage, unlike markers like CRP, for the health care settings especially in LMIC. TLC has the potential to be a robust and important biomarker for general practitioners if confirmed in additional studies in different parts of the world. TLC cut-offs were also dependent on the presence or absence of co-morbidities.

Absolute neutrophil count was found to be an independent risk factor for the prediction of outcomes in COVID-19. There is some evidence that neutrophils boost antiviral defenses via interaction with other immune cells by cytokine discharge, degranulation, and NETs (neutrophil extracellular traps) [45, 46]. In our study, we found a mean ANC of 11710 cells/mm^3 in patients who died due to COVID-19 (normal range: 2500–6000). Similar observations were seen in other studies [47–49]. The cut-off for ANC in those without comorbidities was found to be 13110 cells/mm^3 for predicting mortality while those with a CCI score between 1 and 4 were 11420 cells/mm^3 and cut-off in patients with a CCI score > 5 was 6650 cells/mm^3.

We observed that platelet counts were in the lower end of the normal range both among patients who had severe disease and those who succumbed to the illness. Similar findings were observed in various other studies from a systematic review [50]. Studies done by Georges et al and Shang et al. [51, 52] showed that thrombocytopenia was an independent predictor of mortality for severe community-acquired pneumonia and COVID-19. However, our study did not show statistically significant differences concerning mortality in COVID-19 patients with and without co-morbidities. Similarly, we did not see any statistical significance for other hematological parameters such as Hb, PCV, PLR, and RDW with mortality of COVID-19.

This is the first study to our knowledge to have assessed the effect of various co-morbidities using a validated assessment tool, the CCI on various hematological biomarkers in severe COVID 19 patients. The university teaching hospital and its laboratory are accredited and follow standard operating procedures. The sample size was adequate to identify significant differences between different biomarkers. There are however a few notable limitations to the study. This study was retrospective in nature and data obtained was from a single center. Prospective studies with larger patient enrollment, from multiple centers, would be useful to establish the role of the above-mentioned markers in COVID-19.

Conclusions

In conclusion, in severe COVID-19 patients, CRP was the most reliable biomarker to predict mortality followed by NLR, dNLR, and TLC. We have identified the cut-offs and prognostic power for these biomarkers were different depending on the presence, type, and the number of comorbidities (CCI), on admission to the hospital.

Conflict of interest

None declared.

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