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Early hematological indicators of severe COVID-19 disease in hospitalized patients: Data from a South Asian population

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Abstract

Introduction: Outbreak of corona virus disease in 2019 (COVID-19) has resulted in significant morbidity and mortality worldwide. Our aim is to document hematological parameters of patients with COVID-19 during initial stage of diagnosis and to identify early hematological indicators of severe infection.

Materials and methods: This retrospective study was conducted at Shifa International Hospital, Pakistan from April to November 2020. Patients hospitalized with COVID-19, diagnosed on RT-PCR and had a complete blood count (CBC) done within 48 hours of diagnosis were included. Data was analyzed using IBM® SPSS Statistics.

Results: A total of 425 patients were included in this study out of whom 272(64%) were males. The mean age was 55.61 ± 17.84 years. 95 patients (22.4%) had normal blood counts within 48 hours of COVID-19 diagnosis. Cytopenias were seen in 193(45.4%) patients. There were 75(17.6%) mortalities during the study period. Chi-square test showed that thrombocytopenia, lymphopenia and neutrophilic leucocytosis were significantly associated with mortality ($P = .037$, $P < .001$, $P < .001$ respectively) and need for ventilator ($P = .009$, $P < .001$, $P < .001$, respectively). Neutrophilia was also associated with development of Acute Respiratory Distress Syndrome ($P < .001$). On ROC analysis, Neutrophil-to-Lymphocyte Ratio yielded an area under the curve (AUC) of 0.693 and 0.660 for the outcomes mortality and need for ventilator, respectively. For a subset of 288 patients who had D-dimer levels checked within 48 hours of COVID-19 diagnosis, the AUC for mortality and ventilator need was 0.708 and 0.671, respectively.

Conclusion: Hematological indices are vital indicators in the prognosis and risk stratification of COVID-19 during initial stages of disease.

KEYWORDS

biomarker, COVID-19, diagnosis, lymphopenia, mortality

1 | INTRODUCTION

The outbreak of a novel corona virus causing corona virus disease in 2019 (COVID-19) was officially declared a pandemic by the World Health Organization in March 2020.[1] With over 80 million cases documented till December 2020, COVID-19 is a significant cause of morbidity and mortality worldwide.[2] Laboratory confirmation of COVID-19 infection is through real-time polymerase chain reaction (RT-PCR) which is considered the gold standard diagnostic method in several studies.[3,4]

The clinical manifestations of COVID-19 are diverse, ranging from asymptomatic carrier state to life threatening pneumonia.[5] Early identification and risk stratification of patients with COVID-19 is essential to facilitate timely isolation protocols and patient management.[6] Currently, many studies are investigating the hematological and chemical parameters in patients with COVID-19 in order to identify common patterns and biomarkers seen in this disease.[7,8]

The complete blood count (CBC) is one of the initial investigations ordered when a patient presents to the hospital with any symptoms. This is a quick, simple, and easily available test which offers valuable information about the patient's condition along with severity of signs and symptoms. Previously, the CBC has served as a useful marker in dengue virus infections, with presence of plasmacytoid lymphocytes and thrombocytopenia serving as indicators of diagnosis and severity of dengue infection.[9] Now, a variety of hematological manifestations of SARS-CoV-2 ranging from cytopenias to coagulopathy are also being discovered, which can be also used for disease prognostication in patients with COVID-19.[10]

Therefore, the aim of this study is to document the hematological parameters of patients with COVID-19 during the initial stage of diagnosis. We hope to identify the early hematological indicators of SARS-CoV-2 infection and their association with adverse outcomes in this population of South Asian patients hospitalized with COVID-19.

2 | MATERIALS AND METHODS

This retrospective study was conducted at Shifa International Hospital (SIH), a 550 bed tertiary care hospital in Pakistan which is accredited by the Joint Commission International. The medical records of patients who were hospitalized with COVID-19 during the study period of April to November 2020 were assessed. We included those hospitalized patients in whom SARS-CoV-2 was detected by RT-PCR, whereas patients diagnosed with COVID-19 on radiological imaging alone were excluded. COVID-19 patients managed on an out-patient basis were also excluded from the study population.

A standardized data collection form was developed to retrospectively retrieve relevant information from the medical records. Data were collected regarding patient demographics and laboratory parameters within 48 hours of PCR diagnosis of COVID-19. The outcome variables indicating disease severity were occurrence of all-cause in hospital mortality, need for mechanical ventilation, and length of hospital stay. We also identified those patients who developed Acute Respiratory Distress Syndrome (ARDS), defined according to Berlin criteria.[11]

Hematological parameters were reported according to standardized reference ranges (quoted in the results section). The following definitions were used during interpretation of CBC:

NLR: The neutrophil-to-lymphocyte ratio was determined by dividing the absolute neutrophil count by the absolute lymphocyte count. Normal NLR in healthy adults is up to 3.5, with higher values associated with infection and inflammation.[12]

Lymphopenia: Lymphopenia was defined as an absolute lymphocyte count of less than 1000/μL.[13]

Neutrophilic leucocytosis: Diagnosed as an absolute neutrophil count greater than 8000/μL.[14]

Thrombocytopenia: Defined as platelet count of less than 150 000/μL.

Data were analyzed using IBM® SPSS Statistics Version 21. Mean ± standard deviation were calculated for normally distributed quantitative variables, median, and interquartile range were determined for skewed quantitative variables and frequencies and percentages were reported for qualitative data.[15] Independent samples *t* test, Chi-square test and Receiver Operating Characteristic (ROC) Curves were used to identify the hematological indicators associated with severe COVID-19 disease. *P* value of <.05 was considered significant for all data analysis.

This study was commenced after receiving ethical approval from the Institutional Review Board of SIH. No funding was required for this study and none of the authors have any conflicts of interest to declare.

3 | RESULTS

A total of 425 patients who were hospitalized with COVID-19 and had a CBC done within 48 hours of diagnosis were included in this study. 272 (64%) of the study participants were males, whereas 153 (36%) were females. The mean age was 55.61 ± 17.84 years (range 10 months to 99 years). Average hemoglobin levels of the study population were 12.38 ± 2.61 g/dL, total leucocyte count was 11 701.20 ± 7826.55/μL and mean platelet count was 231 291.47 ± 124 929.54/μL.

Overall, only 95 out of 425 patients (22.4%) had a normal CBC within 48 hours of being diagnosed with COVID-19 in which hemoglobin, total leucocyte count and platelet count were within the normal reference range according to age. The remaining 77.6% patients had an abnormal CBC in which counts of at least one cell line were either low or high. Cytopenias were seen in 193 (45.4%) of our patients. Pancytopenia was seen in 15 (3.5%) patients. The mean absolute lymphocyte count was 1447.74 ± 957/μL, with lymphopenia detected in 160 (37.6%) patients. Mean absolute neutrophil count (ANC) was 9676.40 ± 7272.42/μL (ANC range: 89-48 062/μL), with neutrophilic leucocytosis found in 197 (46.4%) patients. The median neutrophil-to-lymphocyte ratio (NLR) was 6.0 (range 0.06-98). Table 1 summarizes the interpretation of various CBC parameters in our study population.

Leucoerythroblastic picture was seen on admission in the peripheral smear of 7 (1.6%) patients diagnosed with COVID-19.

For a subset of patients, ferritin and D-dimer were also available within 48 hours of COVID-19 diagnosis. Serum ferritin (available for 277 out of 425 patients) showed a median of 779 ng/mL (range

4.8-67 19.8 ng/mL). Median D-dimer levels (available for 288 out of 425 patients) were 1.24 mg/dL (range 0.15-50.35 mg/dL).

Among the outcome variables, the median length of hospital stay was 5 days (range 1-52 days). There were 75 (17.6%) mortalities during the study period. 52 (12.2%) of the patients were put on ventilator. Twenty-eight patients developed ARDS, comprising 53.8% of patients who required ventilatory support.

Chi-square test showed that thrombocytopenia, lymphopenia, and neutrophilic leucocytosis were significantly associated with mortality and need for mechanical ventilation. Independent samples *t* test showed that the mean age of COVID-19 patients who expired during hospital stay was significantly greater than the mean age of those who survived (64.12 ± 16.77 vs 53.78 ± 17.55 years, $P < .001$).

TABLE 1 Interpretation of CBC parameters in our study population

CBC findings	Frequency (%)
Normal CBC	95 (22.4)
Neutrophilic leucocytosis	197 (46.4)
Lymphopenia	160 (37.6)
Cytopenias	193 (45.4)
Cytopenia description	
Thrombocytopenia	39 (9.2)
Anemia	95 (22.4)
Leucopenia	2 (0.5)
Leucopenia + thrombocytopenia	4 (0.9)
Anemia + thrombocytopenia	37 (8.7)
Pancytopenia	15 (3.5)

Abbreviation: CBC, complete blood count.

However, mean age of patients who required ventilator support was not significantly different from those who did not need mechanical ventilation (58.81 ± 15.04 vs 55.16 ± 18.17 years, $P = .168$). Gender was not significantly associated with any adverse outcome. These associations are represented below in Table 2.

Among the subset of patients in whom ARDS was identified, chi-square test showed that the occurrence of neutrophilia within the first 48 hours of COVID-19 diagnosis was the only hematological parameter significantly associated with development of ARDS ($P < .001$).

Spearman's correlation showed that there was a significant association between NLR and length of hospital stay, however this association was weak (Spearman's ρ .237, $P < .001$).

Nonparametric Receiver Operating Characteristic (ROC) analysis was performed to assess the efficacy of various hematological parameters in detecting adverse outcomes associated with COVID-19, specifically mortality and need for mechanical ventilation. NLR yielded an area under the curve (AUC) of 0.693 and 0.660 for the outcomes mortality and need for ventilator, respectively. For the subset of patients who had D-dimer levels checked within 48 hours of hospital admission with COVID-19, the AUC for mortality was 0.708, whereas AUC for ventilator requirement was 0.671. ROC Curves are shown in Figure 1, along with their interpretation in Table 3.

Figure 2 demonstrates the variety of morphological findings seen on peripheral blood smear in our patients.

4 | DISCUSSION

The current COVID-19 pandemic originating in December 2019 in the city of Wuhan, China has widely diffused around the world

TABLE 2 Factors associated with mortality and need for mechanical ventilation

	Mortality			Ventilator		
Variable	Yes N = 75	No N = 350	*P* value	Yes N = 52	No N = 373	*P* value
Age (years) Mean ± SD	64.12 ± 16.77	53.78 ± 17.55	**<.001**	58.81 ± 15.04	55.16 ± 18.17	.168
Gender N (%)						
Male	51 (18.75)	221 (81.25)	.426	33 (12.10)	239 (87.90)	.931
Female	24 (15.69)	129 (84.31)		19 (12.40)	134 (87.60)	
Thrombocytopenia N (%)						
Yes	25 (24.5)	77 (75.5)	**.037**	20 (19.6)	82 (80.4)	**.009**
No	50 (15.5)	273 (84.5)		32 (9.9)	291 (90.1)	
Lymphopenia N (%)						
Yes	43 (26.90)	117 (73.10)	**<.001**	27 (16.90)	133 (83.10)	**<.001**
No	32 (12.10)	233 (87.90)		25 (9.40)	240 (90.60)	
Neutrophilic leucocytosis N (%)						
Yes	50 (25.40)	147 (74.60)	**<.001**	36 (18.30)	161 (81.70)	**<.001**
No	25 (11.00)	203 (89.00)		16 (7.00)	212 (93.00)	

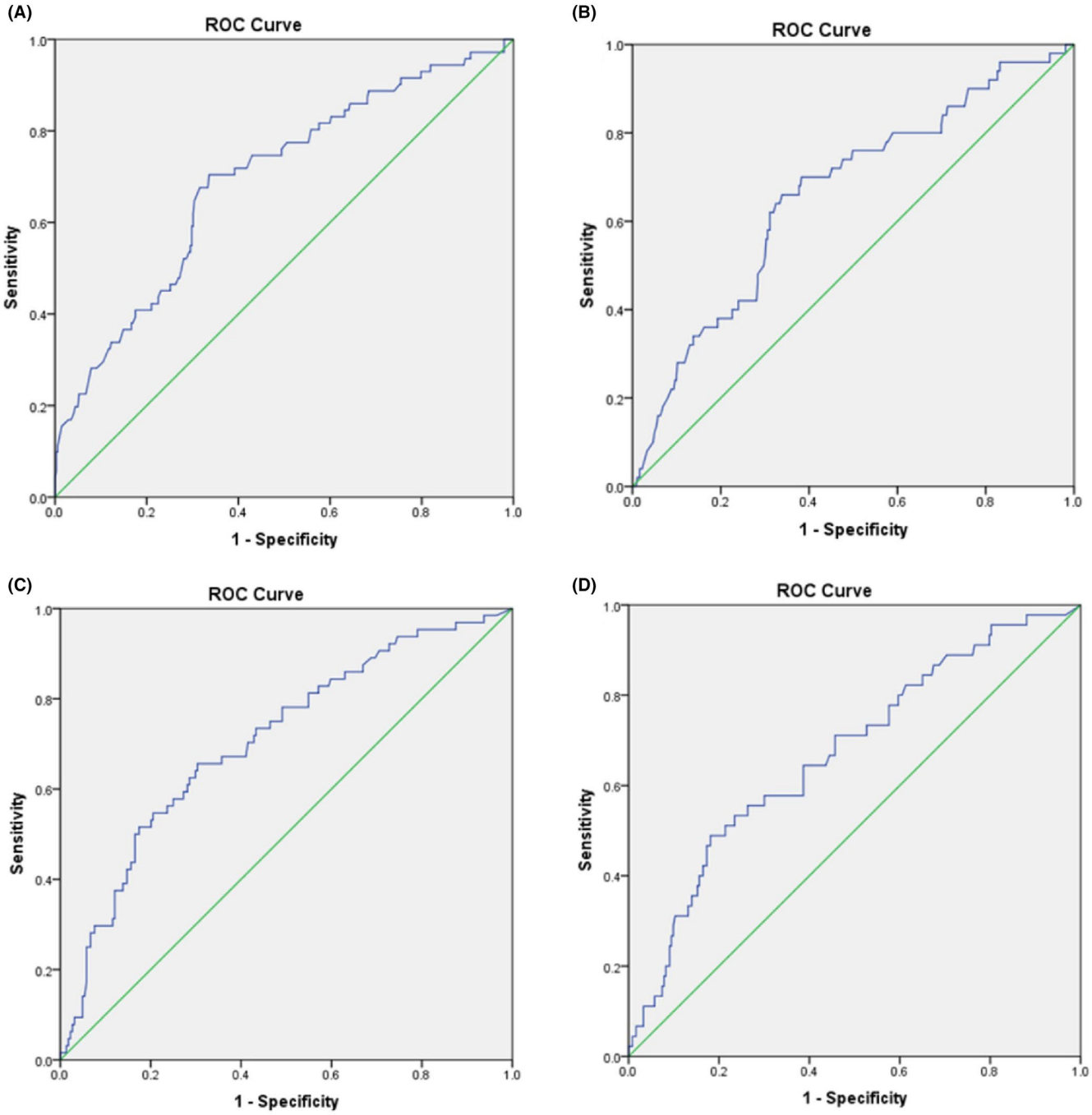

FIGURE 1 ROC curves—(A) NLR for predicting mortality, (B) NLR for predicting need of mechanical ventilation, (C) D-dimer for predicting mortality, (D) D-dimer for predicting need of mechanical ventilation

and has been declared a health emergency by the World Health Organization. Our study reports the spectrum of hematological manifestations during the initial stages of diagnosis of COVID-19 among a South Asian population, as well as their association with adverse outcomes including mortality.

Hematological and biochemical markers are crucial elements in the investigation of suspected COVID-19 cases as their abnormalities can guide clinicians regarding risk stratification and prognosis.[16] CBC is an inexpensive, easy to access and commonly used test, which is recommended at the initial stages of almost all pathologies.

In our study, only 95 (22.4%) patients had a normal CBC within 48 hours of COVID-19 diagnosis. Cytopenias were seen in almost half of the study participants. Another study conducted in China showed that patients of COVID-19 had leukopenia, lymphopenia, and eosinophil cytopenia compared with peripheral film findings of patients who had viral pneumonia due to causes other than COVID-19.[17] It is postulated that the cytopenias occurring in severe

TABLE 3 Efficacy of NLR and D-dimer to predict in-hospital mortality and need for mechanical ventilation

Outcome	Variable	AUC (95% CI)	Sensitivity	Specificity	Cut-off value
Mortality	NLR	0.693 (0.624-0.762)	71.8	60.8	6.625
	D-dimer (mg/dL)	0.708 (0.635-0.780)	70.3	58.5	1.400
Ventilator	NLR	0.660 (0.579-0.741)	70.0	61.7	7.265
	D-dimer (mg/dL)	0.671 (0.585-0.758)	66.7	55.6	1.400

Abbreviation: NLR, neutrophil-to-lymphocyte ratio.

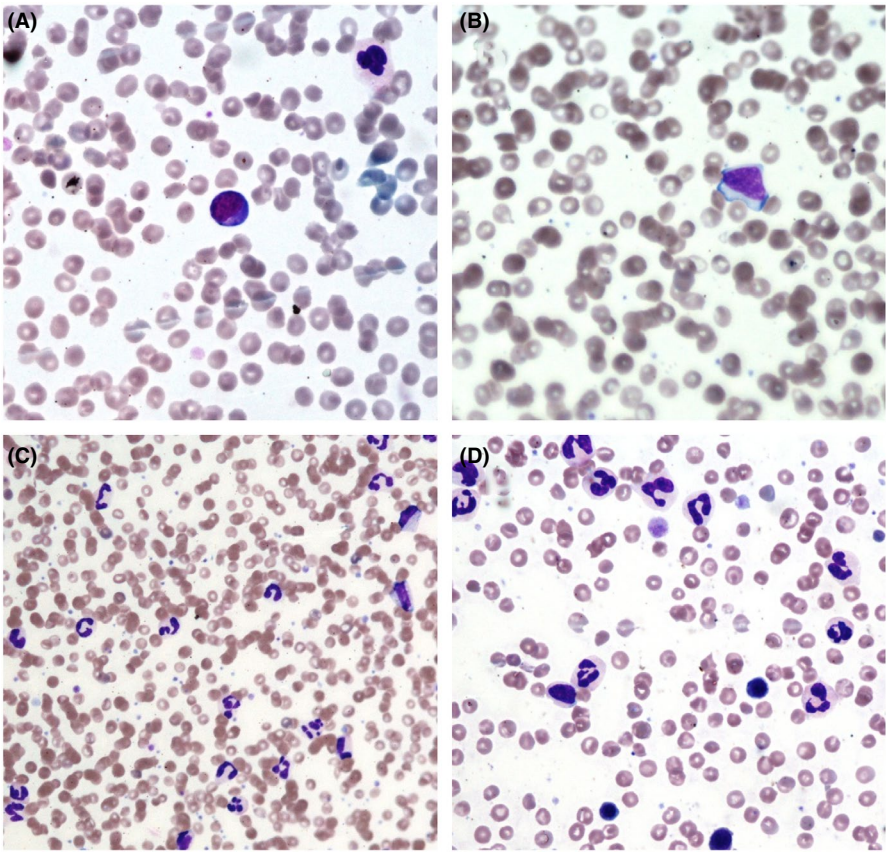

FIGURE 2 Morphological findings in peripheral blood film of COVID-19 patients. (A) Plasmacytoid lymphocyte, (B) Reactive lymphocyte, (C) Neutrophilic leucocytosis, (D) Leucoerythroblastic picture

COVID-19 could be a consequence of increased inflammatory response and cytokine storm resulting in secondary hemophagocytic lymphohistiocytosis.[18] Regarding thrombocytopenia, it is hypothesized that SARS-CoV-2 infection results in increased platelet destruction due to circulating autoantibodies and immune complexes.[19] Lung injury also leads to endothelial damage, platelet activation, and consumption which further worsens thrombocytopenia in COVID-19.[20]

Thrombocytopenia, Lymphopenia, and Neutrophilic leucocytosis were the factors significantly associated with adverse outcomes in our study population. The cutoff value to define lymphopenia has varied in different studies in COVID-19 patients, ranging from <800/μL to <1500/μL.[21] Absolute lymphocyte count of <1000/μL used in our population was significantly associated with both mortality and ventilator need. While we assessed lymphopenia occurring within 48 hours of COVID-19 diagnosis, another study utilizing a time-lymphocyte percentage model found that patients with a lymphocyte percentage of less than 5% detected 17-19 days after COVID-19 diagnosis were also expected to have poor outcomes in terms of mortality and intensive care requirement.[22]

ROC curve analysis was performed in which NLR and D-dimer levels were found to be significant predictors of mortality and need for mechanical ventilation in patients with COVID-19. A study by Zhang et al[23] showed that D-dimer levels on admission greater than 2.0 μg/mL—that is, a fourfold increase from the normal levels—serve as a significant predictor of in-hospital mortality. Another study found that dynamic assessment of D-dimer and NLR throughout hospital stay were indicative of disease prognosis in COVID-19.[24]

Our study is retrospective in nature from a single center in which patients had different hematological presentations such as lymphopenia, neutrophilic leukocytosis, reactive plasmacytoid lymphocytes, and pancytopenia. Hematology laboratory plays an important

role in providing the clinical team with a number of useful diagnostic and prognostic markers.[25]

5 | CONCLUSION

Hematological indices including platelet count, absolute lymphocyte count, neutrophil count, NLR, and D-dimer play a valuable role in diagnosis, risk stratification, and prognosis of patients hospitalized with COVID-19 during early stages of infection.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Fatima Sharif: Study conception and design, data collection, data analysis and interpretation, and manuscript writing. Samreen Khan: Study conception and design, data collection, and manuscript writing. Ayesha Junaid: Study conception and design, and manuscript writing. Sehreen Jahangir, Maria Saeed, and Maira Ijaz: Data collection and manuscript writing. Imran Nazir Ahmad: Study conception and design, and manuscript writing. Shawana Kamran: Manuscript writing.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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