Original Article

Inflammatory biomarkers and adverse outcome in COVID-19: Prelude for future viral pandemics

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Abstract

Background: Dysregulated inflammatory response plays a key role in the pathogenesis of COVID-19. The role of inflammatory markers to predict adverse clinical outcome is still controversial. The aim of this study was to analyze the association of inflammatory markers with disease outcomes independent of the effect of age and co-morbidities. **Materials and Methods:** This is a retrospective analysis of COVID-19 patients admitted at a dedicated COVID center from July 2020 to Mar 2022. Clinical characteristics and inflammatory markers namely serum Ferritin levels, CRP, D-Dimer levels, serum LDH and IL-6 Levels were studied. The following outcome parameters were collected: disease severity at onset and outcome (discharge/death). **Results:** 48.4% of the of 244 COVID-19 cases included had severe disease while 51.6% had moderate disease. Mean age was 61.3 ± 14.17 years and 71.7% were males. Primary Hypertension (48.4%) and Diabetes Mellitus (39.3%) were the most common co-morbidities. Increasing age, smoking, and alcohol consumption were associated with severe disease. CRP, D-dimer, and IL-6 were independent risk factors for disease severity while CRP, D dimer, LDH, Ferritin, and NLR (Neutrophil Lymphocyte ratio) were independent predictors of disease mortality. D-dimer was the most sensitive (95.8%) and specific (92.2%) marker to predict disease severity and serum LDH was the most sensitive (74.7%) to predict disease mortality at baseline. **Conclusion:** Measurement of inflammatory markers might assist clinicians in predicting disease severity and prognosis of COVID-19. This may serve as a benchmark to understand the role of inflammatory markers in other diseases associated with dysregulated inflammatory response.

Keywords: COVID-19, CRP, D-Dimer, IL6, inflammation, LDH, serum ferritin

Introduction

India has reported 45,039,140 confirmed Coronavirus Infectious Disease (COVID) 19 cases till 01 June 2024 with 533,610 deaths. Corresponding figures globally are 775,522,404 confirmed cases with 7,049,617 deaths.[1] According to our current understanding of the natural history of the disease, the majority of infections tend to be mild (80%), requiring no active medical intervention while approximately 20% may go on to develop severe disease requiring hospitalization, need for intensive care unit (ICU) or ventilatory support.[2] While the disease evolved into a pandemic of epic proportions, relentless efforts to understand the pathogenesis highlighted the critical role played by the body's inflammatory response to the viral infection. Thus, a number of studies have dwelled upon the incremental response

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of pro-inflammatory cytokines, C-reactive protein (CRP), D-Dimer, and neutrophil-to-lymphocyte ratio (NLR) to predict adverse clinical outcomes.[3,4] We now know that patients with severe disease requiring ICU care tend to have higher D-dimer levels, increased lactate dehydrogenase (LDH), and serum creatinine values besides higher neutrophil counts and prolonged prothrombin time.[5-7]

Among the most challenging tasks at the height of the pandemic was controlling the viral transmission and, given the fact that we did not have an effective antiviral strategy, based on initial studies, immunomodulation was proposed as an effective therapeutic response. The use of systemic steroids and Interleukin 6 (IL6) inhibitors in this context is, particularly, noteworthy.[8]

Several elegant studies and meta-analyses have emphasized that inflammatory markers increase in COVID-19, particularly, in severe disease.[9-14] However significant shortcomings of these meta-analyses included the fact that they were performed on retrospective studies. The same cohort of patients was included in multiple studies introducing replication. Moreover, publication bias has been reported by several researchers.[15-17]

We now know that age and gender are well-known risk factors for severe COVID-19. Those with co-morbidities like diabetes, obesity, and coronary artery disease are at a higher risk of adverse outcomes.[18-20] The majority of systemic reviews while analyzing the role of inflammatory markers have included patients with multiple co-morbidities who are as such at high risk of adverse disease outcomes, and it is the same set of patients who have higher inflammatory markers in COVID-19.[21-24]

We propose that at this stage, there should be a concerted effort to identify the relevance of each risk factor independently that can cause disease progression and mortality. This will go a long way in recognizing "at-risk" subpopulations, identifying the most sensitive and specific inflammatory markers, and prioritizing healthcare needs. These can thus be utilized as benchmarks in a new pandemic outbreak. The recent surge of "Monkeypox" is an example that we need to prioritize research.

Thus, this study aimed to analyze the association of inflammatory markers with disease outcomes independent of the effect of age, gender, and co-morbidities in patients admitted with moderate and severe COVID-19.

Materials and Methods

This is a retrospective analysis of prospectively collected data of patients admitted at a dedicated tertiary COVID center in Western Maharashtra from July 2020 to Mar 2022.

Inclusion criteria

1. Individuals admitted to the hospital and diagnosed "COVID-19 positive" by reverse transcriptase polymerase chain reaction (RT- PCR)
2. Patients with "moderate" and "severe" disease as per the Indian Council of Medical Research (ICMR) and Ministry of Health and Family Welfare (MOHFW) case definitions of COVID-19.[25]
3. Patients who had complete data records of the clinical profile and had data records of investigations including all of the following inflammatory markers: serum ferritin levels, CRP, D-Dimer levels, serum LDH, and IL-6 Levels done at baseline.

Exclusion criteria

1. Individuals who were asymptomatic or under "mild category" as per ICMR guidelines
2. Incomplete clinical data/record of investigations
3. Individuals who were unwilling to consent.

The institutional ethical clearance for the study was taken before the beginning of the study. All guidelines as per the declaration of Helsinki and good clinical practice guidelines were followed.

Case definitions

We used the ICMR and MOHFW case définitions of COVID-19 for stratification of the cases:[25]

'Asymptomatic or Presymptomatic Infection': Cases who test positive for Severe acute respiratory syndrome-coronavirus (SARS-CoV-2) using a virological test (i.e. RT- PCR) but who have no symptoms that are consistent with COVID-19'.

'Mild Disease': *Cases* who have any of the various signs and symptoms of COVID-19 with positive RT PCR (e.g. fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.

'Moderate disease': Cases who show evidence of lower respiratory disease during clinical assessment or imaging and who had respiratory rate >24/min and oxygen saturation (SpO_2) ≥90 <93% on room air at sea level' with positive RT-PCR.

'Severe disease': Individuals who have SpO_2 <90% on room air at sea level and respiratory rate >30/min, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg, or lung infiltrates >50%' with positive RT-PCR.

"Critical Illness": "Cases who have features of acute respiratory distress syndrome, sepsis or septic shock, and/or multiple organ dysfunction" with positive RT-PCR.

Flow of the study

After obtaining informed consent a detailed relevant history was taken and a physical examination was performed. The following demographic parameters namely date of RT – PCR testing, age, and gender were included. Clinical characteristics included

presenting symptoms, duration of illness, and co-morbidities. All admitted patients underwent complete blood counts, liver function tests, serum albumin levels, renal function tests, and serum prothrombin and activated partial thromboplastin clotting time (aPTT). The following 'inflammatory markers' were studied in all patients: serum ferritin levels, CRP, D-Dimer levels, serum LDH, and IL-6 levels at baseline. The following outcome parameters were collected (presenting symptoms, duration of symptoms before the individual presented for diagnostic RT-PCR, total duration of symptoms, severity of disease at onset, duration of hospital stay, and the final outcome (discharge/death).

Management of COVID 19 cases

Patients with mild and moderate disease were advised home isolation. Those with severe illness and those at high risk were admitted and managed as in-patients. The treatment protocols were regularly updated by the guidelines issued by the ICMR and MOHFW. An institutional committee would meet fortnightly to reassess the treatment protocols, and update and approve any changing treatment guidelines given emerging situations.

Statistics

The data was initially entered into 'Excel sheet' format. 'SPSS Statistic 22.0' (IBM SPSS Statistics, New York, United States) was used for statistical analysis. Descriptive statistics (demography, clinical features at presentation) were calculated. Continuous variables were described by means and standard deviations, while categorical variables were described using frequencies and percentages. Comparisons of continuous variables were performed using independent group *t*-tests when the data were normally distributed, or the Mann–Whitney U test when the data were not normally distributed. Univariate and multivariate Cox regression analyses were conducted to investigate the relation between in-hospital death and levels of inflammatory markers, while Logistics regression analyses were generated to detect the relation between illness status and levels of inflammatory markers. Factors that were significantly associated with primary outcomes in univariate analyses were selected for adjustment while conducting multivariate analyses. Based on the result of regression analyses, Kaplan-Meier survival analyses were used to explore whether inflammatory marker levels were associated with prognosis. All tests were 2-tailed. P <0.05 was considered as significant.

Results

A total of 244 moderate and severe COVID-19 patients with complete clinical records and detailed records of the investigations were included in the present study. 48.4% of cases had severe COVID-19 at the time of admission. There was 100% mortality associated with severe COVID-19 in the present study.

Demographic and clinical characteristics of the cases

The mean age of the case was 61.3 ± 14.17 years with male preponderance constituting 71.7% of the cases of the present study. Primary Hypertension was the commonest comorbidity (48.4%) followed by Diabetes Mellitus (DM) (39.8%) [Table 1]. The commonest symptom at presentation was fever (52%) followed by breathlessness (44.7%). The mean duration of symptoms at presentation was 3.94 days. The detailed demographic and clinical profile of cases is shown in Table 1.

Comparison of clinical and laboratory parameters between Moderate and Severe COVID-19 cases

In a comparison of the baseline characteristics of the "Moderate" and the "Severe" COVID-19 disease [Table 2], we found that increasing age, history of smoking, and alcohol consumption were the factors associated significantly with severe COVID-19 pneumonia. The baseline lab parameters of severity were also compared at the presentation. Elevated D-dimers, elevated CRP, Elevated ESR, Elevated LDH, Elevated Ferritin, and raised IL-6 had significant *P*- values in the case of severe COVID-19 as compared to the Moderate COVID-19 group as shown in Table 2.

Elevated inflammatory markers as an independent risk of severity due to COVID-19

The risk factors for severe COVID-19 were detected by univariate and multivariate logistic regression analysis. Neutrophil count was excluded as it has a co-linear relation with leucocytes. In univariate analysis WBC (HR-2.23, 95% CI: 1.33, 3.72; $P = 0.002$), CRP (HR-2.82, 95% CI: 1.34, 4.56; $P < 0.001$), D-dimer (HR-3.64, 95% CI: 2.45, 6.66; $P < 0.001$), LDH (HR-4.82, 95% CI: 2.72, 8.62; $P < 0.001$), Ferritin (HR-5.84, 95% CI: 3.24, 9.47; $P < 0.001$), and IL-6 (HR-3.84, 95% CI: 2.22, 6.42; $P < 0.001$) were factors

Table 1: Demographic and clinical characteristics of patients

Demographic and clinical characteristics of the cases	Frequency (n=244)
Age	61.31 (15-95) years
Male sex	175 (71.7%)
Female sex	69 (28.3%)
Diabetes mellitus	97 (39.8%)
Primary hypertension	118 (48.4%)
CAD	46 (18.9%)
CKD	17 (7%)
Lung disease	17 (7%)
Chronic liver disease	16 (6.6%)
Malignancy	22 (9%)
Fever	127 (52%)
Cough	72 (29.5%)
Myalgia	33 (13.5%)
Vomiting	7 (2.9%)
Sore throat	10 (4.1%)
Shortness of breath	109 (44.7%)
Diarrhoea	3 (1.2%)
CNS (Altered mental status)	12 (4.9%)
Moderate catageory at admission	126 (51.6%)
Severe catageory at admission	118 (48.4%)
Mean duration of symptoms	3.94 (1-21) days
Median duration of symptoms	3 days

Table 2: Comparison of clinical and lab characteristics of moderate and severe COVID-19 disease

Baseline character	Group of patients		P
	Moderate (*n*=126)	Severe (*n*=118)	
Age	59.12±1.35	63.78±12.69	0.008
Female	34	37	0.671
Hypertension	56	62	0.249
Diabetes mellitus	45	52	0.193
Chronic kidney disease	7	10	0.452
Coronary artery disease	20	26	0.253
Chronic liver disease	6	10	0.304
Anemia	46	39	0.571
Prior respiratory disease	8	9	0.803
History of cancer	8	14	0.179
Smoking	56	68	0.039
Alcohol	32	44	0.045
Median duration of symptoms (days)	3.94±2.30	3.95±2.71	0.969
Hemoglobin (mg/dl)	12.68±1.87	12.63±2.19	0.99
Total leucocyte count (/cumm)	8763±7124	9444±5244	0.527
Polymorph (%)	71.79±13.2	74.58±12.70	0.095
Lymphocyte (%)	17.25±11	15.16±10.36	0.131
NLR	8.595±10.91	9.943±11.77	0.354
Platelet (Lakh/cumm)	2.63±1.07	2.59±1.26	0.776
D-dimer (ng/mL)	553.06±438	1494.997±241	<0.001
CRP (mg/dL)	12.32±9.72	31.685±10.24	<0.001
LDH (IU/L)	374.675±135	580±256	<0.001
Ferritin (ng/mL)	371.235±225	672±332	<0.001
IL-6 (pg/mL)	48.898±23	86.98±42	<0.001
Serum albumin (mg/dL)	3.235±0.95	8.32±0.58	0.331

found to have statistically significant association with severe COVID-19. Subsequently, we took risk factors with *P*- value < 0.1 for multivariate logistic regression analysis and out of these indicators we found that CRP (HR-0.34, 95% CI: 0.14, 0.82; *P* = 0.006), D-dimer (HR-2.85, 95% CI: 1.24, 5.64; *P* < 0.001) and IL-6 (HR-1.54, 95% CI: 0.66, 3.48; *P* = 0.021) has *P* -values still less than 0.05 suggesting that these three inflammatory markers are independent risk factor for disease severity [Table 3].

Elevated Inflammatory markers as an independent risk factor of mortality in COVID-19

We applied the Cox proportion hazard model to evaluate the effect of inflammatory markers on the survival of these patients. In the univariate analysis, we found that elevated WBC (HR-1.74, 95% CI: 1.02, 2.96; *P* = 0.038), elevated CRP (HR-2.43, 95% CI: 1.42, 4.11; *P* = 0.001), elevated D-dimer (HR-3.78, 95% CI: 2.15, 6.66; *P* < 0.001), elevated LDH (HR-4.98, 95% CI: 2.80, 8.85; *P* < 0.001), elevated Ferritin (HR-5.95, 95% CI: 3.35, 10.57; *P* < 0.001), and elevated IL-6 (HR-3.76, 95% CI: 2.17, 6.50; *P* < 0.001) were significantly associated mortality due to COVID-19. Further, we took markers with *P* values < 0.1 into multiple regression analysis and found that elevated CRP (HR-0.28, 95% CI: 0.10, 0.78; *P* = 0.015), elevated D-dimer (HR-2.56, 95% CI: 1.22, 5.85; *P* = 0.026), Elevated LDH (HR-3.36, 95% CI: 1.52, 7.39; *P* = 0.003), and elevated ferritin levels (HR-3.28, 95% CI: 1.56, 6.90; *P* = 0.002) had *P* values less than 0.05, suggesting that these inflammatory biomarkers are independent predictor of mortality due to COVID-19 [Table 4].

Table 3: Univariate and multivariate logistic regression analyses of risk factors for the severity due to COVID-19

Univariate and multivariate logistic regression analysis of risk factors for severity of COVID-19				
Variables	Univariate logistic regression		Multivariate logistic regression	
	P	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)
WBC	0.002	2.23 (1.33–3.72)	0.455	1.59 (0.47–5.38)
Neutrophils	0.029	1.75 (1.05–2.09)	0.326	0.56 (0.20–1.56)
NLR	0.288	1.31 (0.79–2.18)	0.312	0.42 (0.06–2.42)
CRP	<0.001	2.82 (1.34–4.56)	0.006	0.34 (0.14–0.82)
D-Dimer	<0.001	3.64 (2.45–6.66)	<0.001	2.85 (1.24–5.64)
LDH	<0.001	4.82 (2.72–8.62)	0.701	3.48 (1.44–7.84)
Ferritin	<0.001	5.84 (3.24–9.47)	0.666	3.45 (1.46–6.82)
IL-6	<0.001	3.84 (2.22–6.42)	0.021	1.54 (0.66–3.48)

Role of inflammatory biomarkers in predicting severity and mortality due to COVID-19

In statistical analysis we performed receiver operating curve (ROC) for various lab parameters to assess their role in predicting severity and mortality due to COVID-19 disease [Figure 1 and 2]. We found that D-dimer was the most sensitive (95.8%) and specific (92.2%) marker as to predict disease severity. Other inflammatory markers with sensitivity > 80% were CRP, LDH, and IL-6. For prediction of disease-related mortality: NLR, D dimer, LDH, Ferritin, and IL-6 had sensitivity ranging from 60% to 75% and specificity ranging from 50% to 70% in predicting the mortality due COVID-19. At a cutoff of 524.1 ng/dL, D-dimer with an area under the curve (AUC) of 0.722 which has a sensitivity of 73.6% and specificity of 57.9% in predicting disease mortality. Similar values of LDH and serum ferritin have been mentioned in Table 5.

Discussion

The present study includes one of the largest sample sizes from the western part of India. Analysis of the epidemiological and clinical profile of patients was similar to other hospital-based studies from India.[26-29] Our study revealed increasing age, history of smoking, and alcohol consumption as factors associated with increased disease severity. This is consistent with other studies from India and other parts of the world.[30-32]

The primary objective of the study was to assess the role of inflammatory markers to predict disease outcomes in COVID-19, independent of age and co-morbid illness, hence, we focused primarily on inflammatory markers.

CRP

Studies have highlighted that CRP is significantly elevated in the initial inflammatory phase of COVID-19.[33] CRP has been

Table 4: Univariate and multivariate regression analyses of risk factors for the mortality due to COVID-19

Univariate and multivariate cox regression analysis for risk factors for mortality due to COVID-19				
Variables	**Univariate logistic regression**		**Multivariate logistic regression**	
	P	**Hazard ratio (95% CI)**	***P***	**Hazard ratio (95% CI)**
WBC	0.038	1.74 (1.02–2.96)	0.782	1.09 (0.58–2.07)
Neutrophils	0.281	1.35 (0.78–2.32)	0.264	0.56 (0.20–1.56)
NLR	0.004	2.17 (1.26–3.73)	0.027	3.12 (1.14–8.53)
CRP	0.001	2.43 (1.42–4.11)	0.015	0.28 (0.10–0.78)
D-Dimer	<0.001	3.78 (2.15–6.66)	0.026	2.56 (1.22–5.85)
LDH	<0.001	4.98 (2.80–8.85)	0.003	3.36 (1.52–7.39)
Ferritin	<0.001	5.95 (3.35–10.57)	0.002	3.28 (1.56–6.90)
IL-6	<0.001	3.76 (2.17–6.50)	0.216	1.66 (0.74–3.73)

postulated to be an "independent predictor" of disease severity in COVID-19.[34] It has a significant correlation with the risk of thrombotic complications. Individuals with co-morbid diseases such as obesity and type 2 diabetes and elevated CRP have a worse prognosis in COVID-19 possibly highlighting the incremental inflammatory aspects related to hosts' immune response[24,35,36] Meta-analyses and systemic reviews have provided valuable evidence of positive co-relation of CRP levels with severe disease. Huang *et al.*[37] (25 studies, 5350 patients), have reported a risk ratio (RR) of 1.84 (1.45, 2.33), p <0.001 for cumulative poor outcomes with elevated CRP. Yamada *et al.*[38] have included 18 studies and 3278 patients and have concluded that elevated CRP is associated with worse outcomes Odd's ratio (OR) 11.97, 95% Confidence interval (CI): 4.97-28.8. Sahu *et al.*[39] (16 studies, 1896 survivors and 849 non-survivors) have shown a statistically significant association of CRP with adverse outcomes in

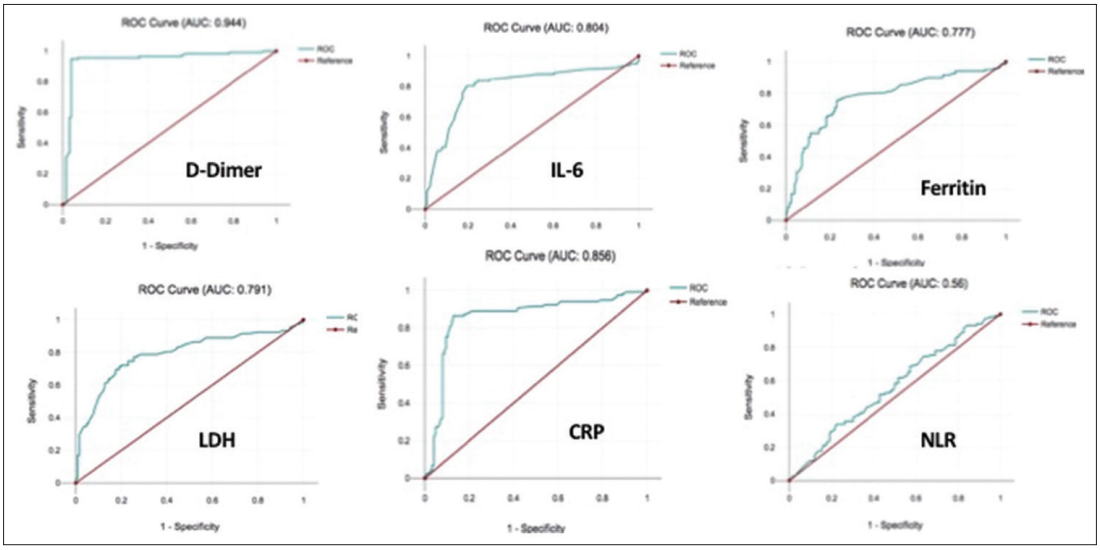

Figure 1: Receiver operating characteristic (ROC) curve for predicting Severity due to COVID by Inflammatory Markers namely D-dimers, IL-6 levels, Serum Ferritin, Serum Lactate dehydrogenase (LDH), C-reactive protein (CRP), and NLR (Neutrophil Lymphocyte ratio) at baseline

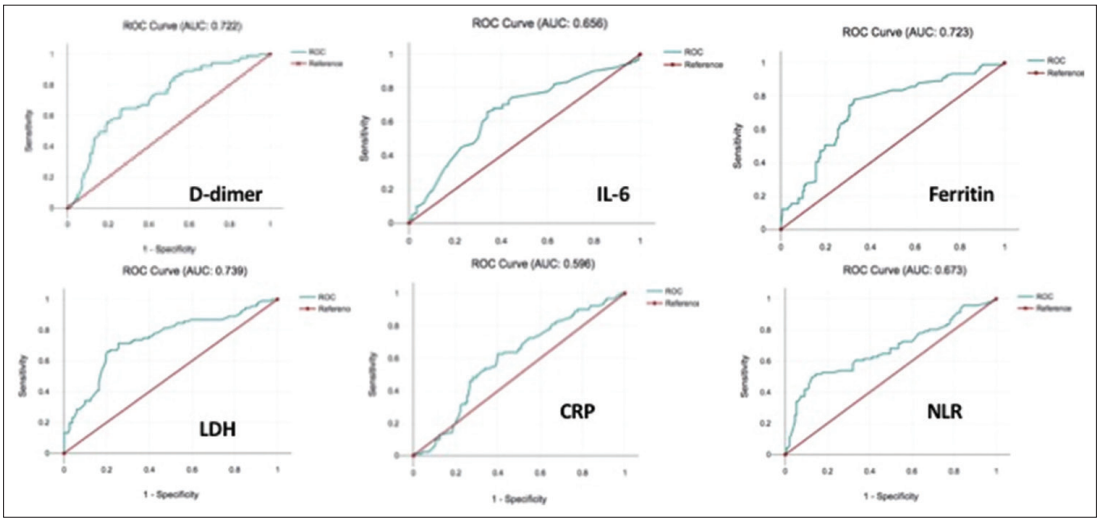

Figure 2: Receiver operating characteristic (ROC) curve for predicting death due to COVID by Inflammatory Markers namely D-dimers, IL-6 levels, Serum Ferritin, Serum Lactate dehydrogenase (LDH), C-reactive protein (CRP), and NLR (Neutrophil Lymphocyte ratio) at baseline

Table 5: Predictors of severity and mortality due to COVID-19

	Predictors of severity due to COVID-19				Predictors of mortality due to COVID-19			
Parameters	**AUC**	**Best cut off**	**Sensitivity (%)**	**Specificity (%)**	**AUC**	**Best cut off**	**Sensitivity (%)**	**Specificity (%)**
WBC	0.6	7650	61	58.7	0.62	7050	62.9	51.3
Neutrophils	0.562	75.5	56.8	57.1	0.648	68.5	67	39.9
NLR	0.56	5.1	56.8	50	0.673	4.69	68.1	50.3
CRP	0.856	27.9	85.6	87.3	0.596	27.95	60.7	59.9
D-dimer	0.944	544.1	95.8	92.9	0.722	524.1	73.6	57.9
LDH	0.791	339.5	80.5	59.7	0.739	375	74.7	62.5
Ferritin	0.777	297	79.7	66.7	0.723	370.5	72.5	69.3
IL-6	0.804	79	80.5	80.2	0.656	83	65.2	66

COVID-19 (Standard difference in means = 1.371, $P = 0.000$). In the index study, CRP was found to be an independent predictor of mortality (HR-0.28, 95% CI: 0.10, 0.78; $P = 0.015$), and severity (HR-0.34, 95% CI: 0.14, 0.82; $P = 0.006$), which is consistent with other published studies.

D-Dimer levels

It is postulated that increased fibrinolysis is positively associated with higher levels of circulating cytokines in COVID-19. The presence of fibrin clots in the endothelial cells increases the expression of IL 8 which further suppresses the time taken for clot lysis and thus predisposes to thrombotic complications seen in COVID-19.[40] Zhao *et al.*[41] in their meta-regression analysis (42 studies, 14,862 patients) have identified 106 variables to be associated with D- Dimer levels in COVID-19. When mild cases were compared to those with severe disease, the mean difference in D-dimer levels was 0.97 μg/mL (95% CI 0.65, 1.29). The authors have reported significant publication bias and have reported D- Dimer levels to be an "independent predictor" of severe disease and associated complications. Similar findings were reported by Li *et al.*[42] (66 studies, 40, 614 patients). Patients with elevated D-dimer levels were associated with poor prognosis [OR = 4.52, 95% CI = (3.61, 5.67), p <0.001; vs. those with low D- dimer levels [HR = 2.81, 95% CI = (1.85, 4.27), p <0.001], Other meta-analyses by Emin Düz *et al.*,[43] Vidali *et al.*[44] and Zhan *et al.*[45] have reported similar findings.

In the present study, D-dimer was found to be an independent predictor of mortality (HR-2.56, 95% CI: 1.22, 5.85; $P = 0.026$) and severity (HR-2.85, 95% CI: 1.24, 5.64; $P < 0.001$). It is the most sensitive and specific marker to predict disease severity in our study at a cutoff value of 544.1 ng/mL. This has therapeutic implications since the increased risk of vascular thrombosis is a well-known complication of COVID-19.[46,47]

Serum ferritin

Coronaviruses including COVID-19 use iron-containing enzymes for regulating replication.[48] Excess iron may also induce a pro-coagulant state by propagating fibrin polymerization.[49] Elevated levels of pro-inflammatory cytokines, metabolic acidosis, and reactive oxygen species cause high ferritin levels in COVID-19. Elevated serum ferritin is thus, an 'independent risk factor' for the severity of COVID-19.[50] Meta-analyses by Cheng *et al.*[51] (52 records, 18 studies, 10, 416 patients) have concluded that those who succumbed to infection had significantly elevated ferritin levels vs. those who survived the illness [Weighed mean difference (WMD) 677.17;95% CI 391.01-963.33, $P < .001$]. Kaushal *et al.*[52] have reported higher levels of serum ferritin in patients with severe or critical disease vs those with mild/moderate disease, [39 studies, standard mean difference (SMD) 0.882;0.738 to 1.026].

In the present study, serum Ferritin was found to be an independent predictor of mortality (HR-3.28, 95% CI: 1.56, 6.90; $P = 0.002$) but does not show a statistically significant association with disease severity. This discrepancy in our findings may have occurred because of the high heterogeneity observed in published meta-analysis which in turn can be explained by the additive effect of other confounders/co-morbid illnesses.[52]

Interleukin 6

The hallmark of severe disease is the marked activation of immune response secondary to COVID-induced inflammation resulting in ARDS and multi-organ failure. This dysregulated immune response is mediated by pro-inflammatory cytokines and chemokines like IL6, IL β, TNF α, IL 18. COVID virus infects the respiratory epithelial cells causing tissue damage and activation of local immune response. Coomes *et al.*[53] in their meta-analyses of 08 studies and 02 preprints have highlighted 2.5-fold increased levels of IL6 in severe COVID-19 versus those with uncomplicated clinical course.

In the present study, Il-6 was found to be an independent predictor of severity (HR-1.54, 95% CI: 0.66, 3.48; $P = 0.021$) but not disease mortality. This finding is particularly interesting as IL6 can induce the production of CRP. In our study, although CRP is associated with disease severity as well as mortality, IL6 is not associated with disease mortality. This is because CRP is produced as a homopentameric protein, often termed as native CRP (n CRP) which transforms into 05 different isomers, i.e. monomeric CRP (m CRP) at the site of inflammation.[54,55] To the best of our knowledge research in COVID-19 has not dwelled into this aspect. Thus, we hypothesize that a particular isoform (m CRP) which is probably associated with an increased risk of thrombosis, is the one giving rise to an increased risk of death in a subset of patients. Another explanation could be the heterogeneity of reported studies.

Serum Lactate dehydrogenase (LDH)

Initial studies evaluating the association of raised LDH with COVID-19 showed a significant co-relation with disease severity. Li *et al.*[56] have predicted that values more than 359.50 IU/L are 93.2% sensitive and 83.2% specific to predict mortality in patients with COVID-19. Martha *et al.*[57] have included 21 studies and 10, 399 patients. They have shown that composite LDH is associated with poor outcomes (OR 5.33 (95% CI 3.90–7.31), $P < 0.001$) and increased mortality (OR 4.22 (95% CI 2.49–7.14) in COVID-19. Interestingly, they reported that diabetes was associated with increased LDH (OR 1.01 (95% CI 1.00 to 1.02), $P = 0.038$) but not age and hypertension. Henry *et al.*[58] (09 studies, 1532 patients) have reported that COVID-19 patients with elevated LDH have 06 fold increased risk of developing severe disease and 16 fold increased risk of mortality.[58] In the present study, Serum LDH was found to be an independent predictor of mortality (HR-3.36, 95% CI: 1.52, 7.39; $P = 0.003$) but not severity due to COVID-19 disease. This finding is again interesting because while multiple published meta-analysis highlights the association with disease severity, none of the studies have analyzed the role of different isozymes of LDH in COVID-19. Reported literature postulates the peculiar role played mu isozyme 3 (present in lung tissue) in those with severe interstitial pneumonia.

Role of other markers in outcome prediction in cases of COVID-19

NLR had a significant role in predicting the severity due to COVID-19 and these are consistent with published literature. In the present study, the role of serum albumin was not found in predicting either severity or mortality due to COVID-19.

Strengths of the study

Our study has a few peculiarities. We have analyzed the role of multiple inflammatory markers in COVID-19 independent of the effect of confounders. This is a variance from other published studies including systemic reviews and meta-analyses. Our sample size is one of the largest among single-center studies that have studied the critical role played by multiple inflammatory markers. Our results have revealed thought-provoking findings especially the fact that while CRP and D- Dimer levels were associated with both the disease severity as well as mortality, IL6 and LDH were associated only with disease severity and mortality respectively. Herein, lies the importance of critically studying the isoform/isozymes of a particular inflammatory marker to better understand their role in disease pathogenesis.

Limitations of the study

Our study has a fair share of limitations. This is a single-centre study with male preponderance. Hence, generalizing these findings to a heterogeneous population would require further research. Ours' is not a follow-up study. We have analyzed our results based on the values of inflammatory markers done at baseline. Therefore, we shall not be able to comment on the variation in the levels of markers and associated clinical profile. These biomarker levels were not blinded to treating physicians. Moreover, the results have to be interpreted in light of the fact that at the onset we did not have a clear understanding of the disease pathogenesis.

Conclusion

Measurement of inflammatory markers might assist clinicians in predicting disease severity and prognosis of COVID-19. This may serve as a benchmark to understand the role of inflammatory markers in other diseases associated with dysregulated inflammatory response.

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Conflicts of interest

There are no conflicts of interest.

References

1. WHO Coronavirus (COVID-19) Dashboard. Available from: https://covid19.who.int.
2. Dos Santos WG. Natural history of COVID-19 and current knowledge on treatment therapeutic options. Biomed Pharmacother 2020;129:110493.
3. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, *et al.* COVID-19: Consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033-4.
4. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, *et al.* Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol 2020;146:128-36.e4.
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al.* Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
6. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al.* China medical treatment expert group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
7. Malik P, Patel U, Mehta D, Patel N, Kelkar R, Akrmah M, *et al.* Biomarkers and outcomes of COVID-19 hospitalisations: Systematic review and meta-analysis. BMJ Evid Based Med 2021;26:107-8.
8. Harrison C. Focus shifts to antibody cocktails for COVID-19 cytokine storm. Nat Biotechnol 2020;38:905-8.
9. Chaudhary R, Garg J, Houghton DE, Murad MH, Kondur A, Chaudhary R, *et al.* Thromboinflammatory biomarkers in COVID-19: Systematic review and meta-analysis of 17,052 patients. Mayo Clin Proc Innov Qual Outcomes 2021;5:388-402.
10. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, *et al.* Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020;180:934-43.
11. Gan J, Li J, Li S, Yang C. Leucocyte subsets effectively predict the clinical outcome of patients with COVID-19 pneumonia: A retrospective case-control study. Front Public Health

2020;8:299.

12. Liu Q, Song NC, Zheng ZK, Li JS, Li SK. Laboratory findings and a combined multifactorial approach to predict death in critically ill patients with COVID-19: A retrospective study. Epidemiol Infect 2020;148:e129.
13. Charostad J, Rezaei Zadeh Rukerd M, Shahrokhi A, Aghda FA, Ghelmani Y, Pourzand P, *et al.* Evaluation of hematological parameters alterations in different waves of COVID-19 pandemic: A cross-sectional study. PLoS One 2023;18:e0290242.
14. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, *et al.* Association of inflammatory markers with the severity of COVID-19: A meta-analysis. Int J Infect Dis 2020;96:467-74.
15. Pires GN, Bezerra AG, Oliveira TB, Chen SFI, Malfatti VDA, Mello VF, *et al.* COVID-19 meta-analyses: A scoping review and quality assessment. Einstein (Sao Paulo) 2021;19:eAO6002.
16. Degarege A, Naveed Z, Kabayundo J, Brett-Major D. Heterogeneity and risk of bias in studies examining risk factors for severe illness and death in COVID-19: A systematic review and meta-analysis. Pathogens 2022;11:563.
17. Boudesseul J, Zerhouni O, Harbert A, Rubinos C. Keeping meta-analyses hygienic during the COVID-19 pandemic. Front Public Health 2021;9:722458.
18. Deng G, Yin M, Chen X, Zeng F. Clinical determinants for fatality of 44,672 patients with COVID-19. Crit Care 2020;24:179.
19. Zhu J, Ji P, Pang J, Zhong Z, Li H, He C, *et al.* Clinical characteristics of 3062 COVID-19 patients: A meta-analysis. J Med Virol 2020;92:1902-14.
20. Li J, Huang DQ, Zou B, Yang H, Hui WZ, Rui F, *et al.* Epidemiology of COVID-19: A systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. J Med Virol 2021;93:1449-58.
21. Liao YH, Zheng JQ, Zheng CM, Lu KC, Chao YC. Novel molecular evidence related to COVID-19 in patients with diabetes mellitus. J Clin Med 2020;9:3962.
22. McNeill JN, Lau ES, Paniagua SM, Liu EE, Wang JK, Bassett IV, *et al.* The role of obesity in inflammatory markers in COVID-19 patients. Obes Res Clin Pract 2021;15:96-9.
23. Mozafari N, Azadi S, Mehdi-Alamdarlou S, Ashrafi H, Azadi A. Inflammation: A bridge between diabetes and COVID-19, and possible management with sitagliptin. Med Hypotheses 2020;143:110111.
24. Chiappetta S, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients with obesity. Int J Obes (Lond) 2020;44:1790-2.
25. MoHFW. Available from: https://www.mohfw.gov.in/.
26. Mohan A, Tiwari P, Bhatnagar S, Patel A, Maurya A, Dar L, *et al.* Clinico-demographic profile and hospital outcomes of COVID-19 patients admitted at a tertiary care centre in north India. Indian J Med Res 2020;152:61-9.
27. Soni SL, Kajal K, Yaddanapudi LN, Malhotra P, Puri GD, Bhalla A, *et al.* Demographic and clinical profile of patients with COVID-19 at a tertiary care hospital in north India. Indian J Med Res 2021;153:115-25.
28. Kundu S, Chauhan K, Mandal D. Survival analysis of patients with COVID-19 in India by demographic factors: Quantitative study. JMIR Form Res 2021;5:e23251.
29. Jain S, Raval DA, Mitra A, Chaudhary D, Khare U. Epidemiological and clinical profile of COVID-19 patients admitted in a Tertiary Care Hospital in Western India. Indian J Community Med 2022;47:138-41.
30. Wolff D, Nee S, Hickey NS, Marschollek M. Risk factors for Covid-19 severity and fatality: A structured literature review. Infection 2021;49:15-28.
31. Gallo Marin B, Aghagoli G, Lavine K, Yang L, Siff EJ, Chiang SS, *et al.* Predictors of COVID-19 severity: A literature review. Rev Med Virol 2021;31:1-10.
32. Chidambaram V, Tun NL, Haque WZ, Majella MG, Sivakumar RK, Kumar A, *et al.* Factors associated with disease severity and mortality among patients with COVID-19: A systematic review and meta-analysis. PLoS One 2020;15:e0241541.
33. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci 2020;57:389-99.
34. Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, *et al.* Prognostic value of C-reactive protein in patients with Coronavirus 2019. Clin Infect Dis 2020;71:2174-9.
35. Al-Samkari H, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, *et al.* COVID-19 and coagulation: Bleeding and thrombotic manifestations of SARS-CoV-2 infection. Blood 2020;136:489-500.
36. Alamdari NM, Afaghi S, Rahimi FS, Tarki FE, Tavana S, Zali A, *et al.* Mortality risk factors among hospitalized COVID-19 patients in a major referral center in Iran. Tohoku J Exp Med 2020;252:73-84.
37. Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: A meta-analysis. Ther Adv Respir Dis 2020;14:1753466620937175.
38. Yamada T, Wakabayashi M, Yamaji T, Chopra N, Mikami T, Miyashita H, *et al.* Value of leukocytosis and elevated C-reactive protein in predicting severe coronavirus 2019 (COVID-19): A systematic review and meta-analysis. Clin Chim Acta 2020;509:235-43.
39. Sahu BR, Kampa RK, Padhi A, Panda AK. C-reactive protein: A promising biomarker for poor prognosis in COVID-19 infection. Clin Chim Acta 2020;509:91-4.
40. Bester J, Matshailwe C, Pretorius E. Simultaneous presence of hypercoagulation and increased clot lysis time due to IL-1β, IL-6 and IL-8. Cytokine 2018;110:237-42.
41. Zhao R, Su Z, Komissarov AA, Liu SL, Yi G, Idell S, *et al.* Associations of D-dimer on admission and clinical features of COVID-19 patients: A systematic review, meta-analysis, and meta-regression. Front Immunol 2021;12:691249.
42. Li Y, Deng Y, Ye L, Sun H, Du S, Huang H, *et al.* Clinical significance of plasma D-dimer in COVID-19 mortality. Front Med (Lausanne) 2021;8:638097.
43. Düz ME, Balcı A, Menekşe E. D-dimer levels and COVID-19 severity: Systematic review and meta-analysis. D-dimer seviyeleri ve COVID-19 şiddeti: Sistematik Derleme ve Meta-analiz. Tuberk Toraks 2020;68:353-60.
44. Vidali S, Morosetti D, Cossu E, Luisi MLE, Pancani S, Semeraro V, *et al.* D-dimer as an indicator of prognosis in SARS-CoV-2 infection: A systematic review. ERJ Open Res 2020;6:00260-2020.
45. Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H, *et al.* Diagnostic value of D-dimer in COVID-19: A meta-analysis and meta-regression. Clin Appl Thromb Hemost 2021;27:10760296211010976.
46. Conte G, Cei M, Evangelista I, Colombo A, Vitale J,

Mazzone A, *et al.* The meaning of D-dimer value in Covid-19. Clin Appl Thromb Hemost 2021;27:10760296211017668.

47. Lehmann A, Prosch H, Zehetmayer S, Gysan MR, Bernitzky D, Vonbank K, *et al.* Impact of persistent D-dimer elevation following recovery from COVID-19. PLoS One 2021;16:e0258351.
48. Liu W, Zhang S, Nekhai S, Liu S. Depriving iron supply to the virus represents a promising adjuvant therapeutic against viral survival. Curr Clin Microbiol Rep 2020;7:13-9.
49. Colafrancesco S, Alessandri C, Conti F, Priori R. COVID-19 gone bad: A new character in the spectrum of the hyperferritinemic syndrome? Autoimmun Rev 2020;19:102573.
50. Lin Z, Long F, Yang Y, Chen X, Xu L, Yang M. Serum ferritin as an independent risk factor for severity in COVID-19 patients. J Infect 2020;81:647-79.
51. Cheng L, Li H, Li L, Liu C, Yan S, Chen H, *et al.* Ferritin in the coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. J Clin Lab Anal 2020;34:e23618.
52. Kaushal K, Kaur H, Sarma P, Bhattacharyya A, Sharma DJ, Prajapat M, *et al.* Serum ferritin as a predictive biomarker in COVID-19. A systematic review, meta-analysis and meta-regression analysis. J Crit Care 2022;67:172-81.
53. Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: A systematic review and meta-analysis. Rev Med Virol 2020;30:1-9.
54. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol 2018;9:754.
55. Wang X, Tang G, Liu Y, Zhang L, Chen B, Han Y, *et al.* The role of IL-6 in coronavirus, especially in COVID-19. Front Pharmacol 2022;13:1033674.
56. Li C, Ye J, Chen Q, Hu W, Wang L, Fan Y, *et al.* Elevated Lactate Dehydrogenase (LDH) level as an independent risk factor for the severity and mortality of COVID-19. Aging (Albany NY) 2020;12:15670-81.
57. Martha JW, Wibowo A, Pranata R. Prognostic value of elevated lactate dehydrogenase in patients with COVID-19: A systematic review and meta-analysis. Postgrad Med J 2022;98:422-7.
58. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, *et al.* Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med 2020;38:1722-6.