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*Original Article*

# A study to evaluate the role of biomarkers in assessing the severity of COVID-19

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## ABSTRACT

**Objectives:** COVID-19 is a systemic multiorgan disease caused by severe acute respiratory syndrome coronavirus 2, a beta-type RNA coronavirus. Different laboratory markers are implicated as an indicator of disease severity, progression, and outcome. The objectives of the study are as follows: To study the role of laboratory biomarkers in assessing the severity of COVID-19. (1) To compare the values of various biomarkers (interleukin-6 [IL-6], C-reactive proteins [CRPs], D-Dimer, S. Ferritin) in clinically categorized mild-moderate and severe COVID-19 patients. (2) To compare clinical severity with computed tomography (CT) severity score in COVID-19 patients. (3) To determine association between laboratory markers and CT severity score in COVID-19 patients.

**Materials and Methods:** A hospital-based, retrospective, and observational study was conducted at our tertiary care center on 200 patients to assess the role of different laboratory biomarkers in COVID-19 patients. Values of laboratory markers, serum urea, serum creatinine, serum sodium, and serum potassium were compared between clinically categorized mild/moderate and severe COVID-19 patients. Non-contrast CT chest was performed and CT severity score (mild ≤ 7, moderate 8–17, and severe ≥ 18) was assessed in COVID-19 patients.

**Results:** Levels of total leukocyte count (TLC), D-dimer, CRP, lactate dehydrogenase (LDH), S. ferritin, CK-MB, IL-6, urea, sodium, and potassium were significantly elevated in severe COVID-19 group as compared to mild/moderate group. Chest CT Severity Score ≥18 was found in 37.1% of patients in severe group; while only in 4.2% patients in mild-to-moderate group and chest CT Severity Score and clinical severity of COVID-19 showed statistically significant agreement ($P < 0.001$). CRP, LDH, NTPROBNP, S. Ferritin, and CPK showed statistically significant positive correlation with CT severity score whereas sodium and potassium levels showed significant negative correlation.

**Conclusion:** Elevated levels of TLC, D-dimer, CRP, LDH, S. ferritin, CK-MB, and IL-6 were associated with severe COVID-19 cases. NTPROBNP, CRP, LDH, D-dimer, IL-6, and S. Ferritin demonstrated better ability to predict the severity of COVID-19 in comparison to other laboratory biomarkers.

**Keywords:** Biomarkers, COVID-19, Interleukin-6, C-reactive proteins, D-dimer, S. Ferritin, Computed tomography

## INTRODUCTION

The coronavirus disease popularly known as the COVID-19 was first reported in Wuhan, Hubei in China in December 2019 and has rapidly evolved from an epidemic outbreak into a global pandemic infecting more than 13 million individuals across the world.[1] Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus has been implicated as the causative agent.[2]

First COVID-19 case in India was reported from Kerala on January 30, 2020, with a positive travel history to Wuhan.

COVID-19 is a systemic multi organ disease caused by SARS-CoV-2, a beta-type RNA coronavirus.[3] It is more contagious than either SARS-CoV (in 2003) or MIDDLE EAST RESPIRATORY SYNDROME-coronavirus (MERS-CoV) (in 2012) but has lower case fatality.[4-6] The virus primarily attacks the lungs, causing drastic lung injury and acute respiratory distress syndrome in severe cases which could be fatal.[7]

Different laboratory markers are implicated as an indicator of disease severity, progression, and outcome. Deranged cell counts, such as anemia, polycythemia, leukopenia, and leukocytosis with neutrophil predominance and decreased platelet count, are found to be associated with severe disease and worse outcome in hospitalized patients.[8,9] Similarly, raised liver enzymes and total bilirubin levels were identified in severe and critical patients.[8,10-12]

Raised inflammatory response of the body as manifested by raised laboratory values of various interleukins and C-reactive proteins (CRPs) is also reported.[13-15] In addition, raised coagulation markers such as fibrinogen and prothrombin time are identified in severe and critical patients.[13,16]

Studies have shown that severe or fatal cases of COVID-19 disease are associated with an elevated white cell count, blood urea nitrogen, creatinine, markers of liver and kidney function, CRP, interleukin-6 (IL-6), lower lymphocyte (<1000/μL), and platelet counts (<100 × $10^9$/L) as well as albumin levels compared with milder cases in which survival is the outcome.[17,18]

Since laboratory medicine has always supported clinical decision making in various infectious diseases, it is important to assess the ability of laboratory-derived biomarkers to facilitate risk stratification of COVID-19 disease. Therefore, in the present retrospective study, we assessed the relationship of various biomarkers with disease severity in hospitalized COVID-19 patients.

## MATERIAL AND METHODS

This hospital-based, retrospective, and observational study was conducted at our tertiary care center on 200 patients to assess the role of different laboratory biomarkers in COVID-19 patients. The data of patients for study were collected over the period of 1 year from August 2020 to July 2021. The study was conducted on COVID-19 patients who fulfilled the inclusion criteria, admitted in COVID ward and intensive care unit from the month of August 2020 to July 2021 in the Department of Pulmonology, Fortis Hospital, Vasant Kunj, New Delhi, a tertiary care center with all the required diagnostic modalities including research facility.

Patients with age ≥18 years and positive reverse transcriptase polymerase chain reaction (RT-PCR) report for COVID were included in the study. Patients with age <18 years and pregnant females were excluded from the study.

After approval from the Institutional Ethics Committee, the data of the patients admitted from August 2020 onward to July 2021 were collected as per study proforma and analyzed.

A total of 200 patients admitted with COVID-19 at our tertiary care center in the Department of Pulmonology, Fortis Hospital, Vasant Kunj, New Delhi, irrespective of gender, race, religion, and socioeconomic background were finally enrolled into the study after excluding those who did not fulfill the inclusion criteria.

Data of detailed history, general physical examination, systemic examination, laboratory parameters, and radiological findings were incorporated as per pro forma.

Demographic and epidemiological statistics, such as age, sex, and disease history, were gathered from the patient records. For laboratory confirmation, real-time RT-PCR was used as gold standard, according to the recommended protocol of the hospital.

The data of all the COVID RT-PCR positive patients admitted at our center were analyzed and the reports of the relevant laboratory investigations were noted. The values of routine investigations and specific biomarkers were recorded. CRP was detected by lactate-pyruvate assay method. Erythrocyte sedimentation rate (ESR) was measured by Westergren's international standard method. For D-dimer immunoturbidometry by photometry method was used. For S. Ferritin chemiluminescent microparticle immunoassay was used and for IL6, enzyme-linked immunosorbent assay method was used.

Values of laboratory markers (total blood count, D-dimer, CRP, lactate dehydrogenase [LDH], NTPROBNP, CPK, CKMB, IL-6, and ferritin), serum urea, serum creatinine, serum sodium, and serum potassium were compared between clinically categorized mild/moderate and severe COVID-19 patients. Non-contrast computed tomography (CT) chest was performed and CT severity score (mild ≤ 7, moderate 8–17, and severe ≥ 18) was assessed in COVID-19 patients.

## RESULTS

The hospital-based, retrospective, and observational study was conducted on 200 COVID-19 positive patients admitted in our tertiary care center in the Department of Pulmonology, Fortis Hospital, Vasant Kunj, New Delhi, from the month of August 2020 to July 2021.

Out of total 200 studied patients; 54.0% of cases were in age group 51–70 years, with mean age 63.14 ± 11.71 years; the minimum study patient's age was 18 years and maximum was 89 years. About 75.5% of patients were males, 78.5% of cases were mild-to-moderate and rest 21.5% of cases were severe. Radiological severity of lung involvement in patients was assessed using CT severity score 60.

Cough (91.0%) and fever (88.0%) were more common symptoms of the COVID-19 patients followed by diarrhea (66.5%), myalgia (65.0%), loss of smell (62.0%), dyspnea (54.5%), sore throat (42.0%), anorexia (29.5%), expectoration (13.0%), and hemoptysis (11.5%).

Out of a total 200 studied patients, 132 (66.0%) patients had the history of comorbidities, diabetes mellitus (55.3%) and hypertension (36.4%) were the most common comorbidities followed by AKI (9.8%), CLD (7.6%), CKD (6.8%), and CAD in 3.0% of patients.

The severity of COVID-19 was assessed as per the Ministry of Health and Family Welfare guidelines, Government of India. There were 105 (52.5%) patients in severe group and 95 (47.5%) in mild-to-moderate group [Table 1].

It was observed that mean age in severe group (65.71 ± 11.60) was significantly higher than the mild/moderate group (60.29 ± 11.23) of COVID-19 patients [Table 2].

Fever and hemoptysis as presenting symptoms were significantly higher in severe group but rest other symptoms were comparable in both groups [Table 3].

Patients having the history comorbidity were significantly higher in severe group as compared to mild/moderate group [Table 4].

Levels of TLC, D-dimer, CRP, LDH, S. ferritin, CK-MB, IL-6, urea, sodium, and potassium were significantly elevated in severe COVID-19 group as compared to mild/moderate group [Table 5].

Chest CT severity score ≥18 was found in 37.1% patients in severe group; while only in 4.2% of patients in mild-to-moderate group and chest CT severity score and clinical severity of COVID-19 showed statistically significant agreement ($P < 0.001$) [Table 6 and Graph 1].

CRP, LDH, NTPROBNP, S. Ferritin, and CPK showed statistically significant positive correlation with CT severity score whereas sodium and potassium levels showed significant negative correlation [Table 7].

Receiver operation characteristic (ROC) curves were performed to determine which laboratory biomarkers provided better prediction of disease severity. The markers with higher area under the curve (AUC) values performed better in predicting disease severity [Table 8 and Graph 2].

**Table 1:** Distribution of chest CT severity score in both groups.

| CTSS | Group | | *P*-value* |
|---|---|---|---|
| | Mild-to-moderate (*n*=95) (%) | Severe (*n*=105) (%) | |
| Mild ≤7 | (0.0) | 0 (0.0) | <0.001 |
| Moderate 8–17 | 91 (95.8) | 66 (62.9) | |
| Severe ≥18 | 4 (4.2) | 39 (37.1) | |

*Chi-square test. $P<0.05$ is statistically significant while $P<0.001$ is highly significant.

**Table 2:** Age group distribution in both groups.

| Age group (Years) | Group | | *P*-value |
|---|---|---|---|
| | Mild-to-moderate (*n*=95) (%) | Severe (*n*=105) (%) | |
| 18–40 | 9 (9.47) | 1 (0.95) | 0.043* |
| 41–50 | 13 (13.68) | 10 (10.5) | |
| 51–60 | 28 (29.5) | 26 (24.8) | |
| 61–70 | 25 (26.3) | 29 (27.6) | |
| 71–80 | 15 (15.8) | 27 (25.7) | |
| >80 | 5 (5.3) | 12 (11.4) | |
| Mean±SD | 60.29±11.23 | 65.71±11.60 | <0.001# |

*Chi-square test, #Independent samples *t*-test. $P<0.05$ is statistically significant while $P<0.001$ is highly significant.

**Table 3:** Presenting symptoms distribution in both groups.

| Symptoms | Group | | *P*-value* |
|---|---|---|---|
| | Mild-to-moderate (*n*=95) (%) | Severe (*n*=105) (%) | |
| Fever | 77 (81.1) | 99 (94.3) | **0.005** |
| Cough | 84 (88.4) | 98 (93.3) | 0.225 |
| Expectoration | 12 (12.6) | 14 (13.3) | 0.883 |
| Dyspnea | 54 (56.8) | 55 (52.4) | 0.527 |
| Myalgia | 59 (62.1) | 71 (67.6) | 0.414 |
| Sore throat | 36 (37.9) | 48 (45.7) | 0.263 |
| Hemoptysis | 6 (6.3) | 17 (16.2) | **0.029** |
| Diarrhea | 66 (69.5) | 67 (63.8) | 0.397 |
| Loss of smell | 62 (65.3) | 62 (59.0) | 0.366 |
| Anorexia | 29 (30.5) | 30 (28.6) | 0.762 |

*Chi-square test. $P<0.05$ is statistically significant while $P<0.001$ is highly significant.

## DISCUSSION

As SARS-CoV-2 infection progresses that the lymphocyte count in the blood decreases drastically, along with an increase in the neutrophil count and a decrease in the platelet number, with prolonged activated thromboplastin time and raised CRP, cardiac enzymes, and liver function tests.

**Table 4:** Presenting comorbidity distribution in both groups.

| Comorbidity | Group | | *P*-value* |
|---|---|---|---|
| | Mild-to-moderate (*n*=95) (%) | Severe (*n*=105) (%) | |
| Present | 48 (50.5) | 84 (80.0) | **<0.001** |
| Absent | 47 (49.5) | 21 (20.0) | |
| Present (*n*=132) | | | |
| DM | 26 (54.2) | 47 (56.0) | 0.843 |
| HTN | 16 (33.3) | 32 (38.1) | 0.584 |
| AKI | 5 (10.4) | 8 (9.5) | 0.868 |
| CLD | 7 (14.6) | 4 (4.8) | 0.050 |
| DKA | 3 (6.2) | 7 (8.3) | 0.663 |
| CKD | 4 (8.3) | 5 (6.0) | 0.602 |
| CAD | 2 (4.2) | 2 (2.4) | 0.565 |

DM: Diabetes mellitus, HTN: Hypertension, CRP: C-reactive proteins, *Chi-square test. *P*<0.05 is statistically significant while *P*<0.001 is highly significant.

**Table 5:** Comparison of laboratory biomarkers in both groups.

| Investigations | Group | | *P*-value* |
|---|---|---|---|
| | Mild to Moderate (*n*=95) | Severe (*n*=105) | |
| Hemoglobin (g%) | 12.47±1.66 | 11.88±1.84 | **0.020** |
| TLC ($10^9$/L) | 6.56±2.219 | 17.90±7.87 | **<0.001** |
| D-dimer (ng/mL) | 443.49±121.08 | 610.82±367.40 | **<0.001** |
| CRP (mg/L) | 47.67±45.43 | 91.76±50.54 | **<0.001** |
| LDH (U/L) | 213.42±70.20 | 343.16±83.11 | **<0.001** |
| S. Ferritin (ng/ml) | 427.35±339.71 | 1447.73±939.86 | **<0.001** |
| CPK (mcg/L) | 172.57±209.41 | 231.39±250.54 | 0.512 |
| CK-MB (IU/L) | 21.22±8.73 | 33.16±15.05 | **<0.001** |
| IL6 (pg/mL) | 8.54±4.56 | 20.44±13.39 | **<0.001** |
| Urea (mg/dl) | 20.28±10.11 | 34.44±16.53 | **<0.001** |
| Creatinine (mg/dl) | 1.00±0.32 | 1.21±0.42 | 0.434 |
| Sodium (mEq/L) | 137.60±3.77 | 134.42±5.09 | **<0.001** |
| Potassium (mmol/L | 4.03±0.35 | 4.28±0.75 | **<0.001** |
| NT Pro BNP (pg/ml) | 164.04±102.15 | 445.42±110.33 | **0.571** |

*Independent samples *t*-test, CRP: C-reactive proteins, TLC: Total leukocyte count. *P*<0.05 is statistically significant while *P*<0.001 is highly significant.

Inflammatory cytokine secretion (IL1RA, IL-1B, IL-6, IL-7, and IL-8) is associated with cytokine storm and contributes to the pathogenesis of severe cases of COVID-19.[19] Largely, clinical and laboratory parameters contributing to complications were elderly, dyspnea, decreasing oxygen saturation, elevated aspartate aminotransferase (AST), elevated neutrophil count, gamma-glutamyltransferase levels, and LDH, raised CRP, high serum ferritin level, and elevated IL-6.[20] Specific blood parameters, such as lymphopenia, and certain chemical features, in particular, troponin-I, serum creatinine, alanine aminotransferase (ALT), and alkaline phosphatase (ALP), were delineated to be linked with the severity of COVID-19.[21] Notably, increasing serum CRP levels corresponded to disease progression, serving as an early predictor for COVID-19 complications.

**Table 6:** Distribution of chest CT severity score in both groups.

| CTSS | Clinical severity of COVID-19 | | *P*-value* |
|---|---|---|---|
| | Mild-to-moderate (*n*=95) (%) | Severe (*n*=105) (%) | |
| Mild | | | |
| ≤7 | (0.0) | 0 (0.0) | <0.001 |
| Moderate | | | |
| 8–17 | 91 (95.8) | 66 (62.9) | |
| Severe | | | |
| ≥18 | 4 (4.2) | 39 (37.1) | |

*Chi-square test. *P*<0.05 is statistically significant while *P*<0.001 is highly significant.

**Table 7:** Pearson correlation between laboratory markers and chest CT severity score.

| Laboratory findings | Chest CT severity score | |
|---|---|---|
| | Pearson correlation | *P*-value |
| Hemoglobin (g%) | −0.077 | 0.277 |
| TLC ($10^9$/L) | −0.035 | 0.621 |
| D-dimer (ng/mL) | −0.075 | 0.290 |
| CRP (mg/L) | 0.513** | **<0.001** |
| LDH (U/L) | 0.554** | **<0.001** |
| NT Pro BNP (pg/ml) | 0.495** | **<0.001** |
| S. Ferritin (ng/ml) | 0.326** | **<0.001** |
| CPK (mcg/L) | 0.356** | **<0.001** |
| CK-MB (IU/L) | 0.047 | 0.509 |
| IL-6 (pg/mL) | −0.035 | 0.624 |
| Urea (mg/dl) | 0.118 | 0.097 |
| Creatinine (mg/dl) | 0.368** | **<0.001** |
| Sodium (mEq/L) | −0.401** | **<0.001** |
| Potassium (mmol/L) | −0.247** | **<0.001** |

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed), CRP: C-reactive proteins, TLC: Total leukocyte count, IL-6: Interleukin-6. *P*<0.05 is statistically significant while *P*<0.001 is highly significant.

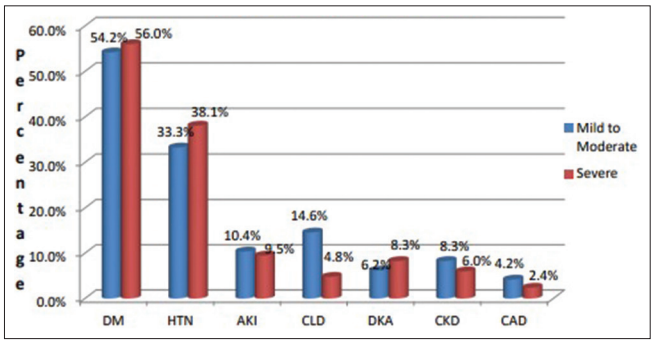
Elevated levels of TLC, D-dimer, CRP, LDH, S. ferritin, CK-MB, IL-6 were significantly associated with severe COVID-19 cases. Kaur *et al.*[22] reported that serum levels of CRP, troponin-I, ALP, ALT, serum creatinine, and ferritin were markedly increased in COVID-19 patients. Melo *et al.*[23] study reported similar findings.

The association between Chest CT Severity Score and laboratory markers was found to be statistically significant in our study. Gupta *et al.*[24] reported the Pearson correlation coefficients calculated and serum LDH showed strongest correlation

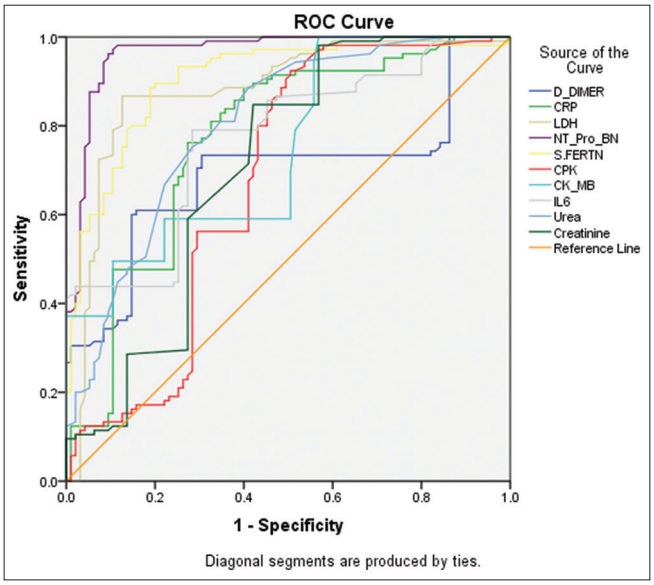
**Table 8:** AUC values of various biomarkers.

| Test result variable(s) | AUC | Std. error[a] | Asymptotic Sig.[b] | Asymptotic 95% confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| D-dimer (ng/mL) | 0.692 | 0.039 | 0.000 | 0.616 | 0.768 |
| CRP (mg/L) | 0.769 | 0.035 | 0.000 | 0.701 | 0.837 |
| LDH (U/L) | 0.879 | 0.027 | 0.000 | 0.827 | 0.931 |
| NT Pro BNP (pg/ml) | 0.965 | 0.013 | 0.000 | 0.939 | 0.990 |
| S. Ferritin (ng/ml) | 0.902 | 0.022 | 0.000 | 0.858 | 0.946 |
| CPK (mcg/L) | 0.675 | 0.041 | 0.000 | 0.595 | 0.754 |
| CK-MB (IU/L) | 0.748 | 0.034 | 0.000 | 0.681 | 0.815 |
| IL6 (pg/mL) | 0.772 | 0.033 | 0.000 | 0.708 | 0.837 |

The test result variable(s): D-DIMER, CRP, LDH, NT Pro BN, S. FERTN, CPK, CKMB, and IL6, has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. [a]Under the nonparametric assumption, [b]Null hypothesis: true area*n*=0.5, CRP: C-reactive proteins. AUC: Area under the curve. *P*<0.05 is statistically significant while *P*<0.001 is highly significant.




**Graph 1:** Comorbidity distribution.




**Graph 2:** ROC curves.

with increasing lung involvement in high-resolution CT chest, followed by serum ferritin. Wang *et al.*[4] reported that significant correlations were found about age, comorbidities, white blood cell count, neutrophil count, lymphocyte count, plasma glucose, serum potassium, albumin, D-dimer, HDL-C, TBIL, AST, ALT, LDH, ESR, SAA, CRP, and PCT. LDH (r = 0.548), D-dimer (r = 0.477), SAA (r = 0.58), and CRP (r = 0.477) were moderately correlated. Strikingly, this study revealed negative correlation between disease severity and lymphocyte count, albumin, serum potassium, and HDL-C.

ROC curves were made to analyze the ability of biomarkers to predict disease severity. The AUC case of D-DIMER for severe patients was 0.692. The AUC in case of CRP for severe patients was 0769. Our findings were supported by the study conducted by Wang *et al.*[4] reported that respective AUC values for IL-6 and CRP in the evaluation cohort were 0.97 and 0.86, and they were similar in the validation cohort (0.90 and 0.83, respectively). The calculated optimal values during the course of disease from the evaluation cohort (IL-6 level > 80 pg/mL and CRP level > 97 mg/L) both correctly classified 80% of patients in the validation cohort regarding their risk of developing respiratory failure.

## CONCLUSION

The history of comorbidity was significantly higher in severe group than the mild-to-moderate patients of COVID-19. Elevated levels of TLC, D-dimer, CRP, LDH, S. ferritin, CK-MB, and IL-6 were associated with severe COVID-19 cases. Chest CT Severity Score and clinical findings of the severity of COVID-19 showed significant agreement. NTPROBNP, CRP, LDH, D-DIMER, IL-6, and S. Ferritin demonstrated better ability to predict the severity of COVID-19 in comparison to other laboratory biomarkers.

### Declaration of patient consent

Patient's consent not required as patient's identity is not disclosed or compromised.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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