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Assessment of diagnostic and prognostic laboratory biomarkers in severe COVID-19 patients admitted to intensive care unit

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Abstract

Coronavirus disease (COVID-19) is a global pandemic. In the first two years of the pandemic, nearly 15 million people died worldwide. Accurate and rapid laboratory diagnosis of COVID-19 infection is one of the milestones of pandemic control. Therefore, this study aimed to compare the diagnostic and prognostic accuracy of mainly used laboratory biomarkers (CRP, ferritin, IL-6, D-dimer, procalcitonin, and LDH) in the sera of severe COVID-19 Egyptian patients, to assess the most appropriate biomarker used in severe COVID-19 patients. A total of 120 COVID-19 patients and 50 normal controls were enrolled into our study. Demographic data, hospitalization time, medical history, oxygen saturation, respiratory rate, oxygen supply, laboratory findings and thorax tomography of the patients were obtained from the hospital electronic information system retrospectively. Our results revealed that the serum levels of CRP, ferritin, IL-6, D-dimer, PCT and LDH were highly significantly increased in severe COVID-19 patients as compared to normal controls (p<0.001), and in non-survivors as compared to survivors (p<0.001). By using ROC curve analysis, IL-6 appeared to be the most sensitive and specific marker with 80.9% sensitivity and 84.9% specificity; followed by LDH with 85.1% sensitivity and 82.8% specificity in the prediction of death. In conclusion CRP and IL-6 could be the most appropriate biomarkers in the diagnosis of severe COVID-19 disease, while IL-6 and LDH may be good predictors of mortality between severe COVID-19 patients.

Keywords: SARS-CoV-2 infection; COVID-19; Procalcitonin; IL-6; LDH; CRP; ferritin; D- dimer.

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and first emerged in Wuhan-China in December 2019, was declared as a global pandemic by the World Health of Organization (WHO) on 11 March 2020.[1,2] It has become a catastrophic public health crisis affecting many people; as till 12 July 2021, there were 187,916,376 confirmed cases and about 4,053,093 deaths, reported in about 221 countries.[3] The clinical presentation of COVID-

19 varies greatly from asymptomatic to milder symptoms, including dry cough, fever, myalgia, dyspnea, sore throat and headache, or even to severe and emergent manifestation including chest pain, confusion, hypoxia, pneumonia and other complications requiring intensive care unit (ICU) admission and mechanical ventilation.[4] Accurate and rapid laboratory diagnosis of COVID-19 infection is one of the milestones of pandemic control.[5]Worldwide, the scientists and researchers have been searching for predictors of COVID-19 disease severity to identify them and then applying the most appropriate medical management. Laboratory biomarkers are faster, easier, and less expensive to obtain. Therefore, they are preferred method to monitor and predict outcomes and prognosis of disease.[6]

The clinical guidelines of WHO define "severe COVID-19" as patients with clinical signs of pneumonia (fever, cough, dyspnea, and fast breathing) accompanied by one of the following: severe respiratory distress; respiratory rate > 30 breaths/min; or O_2 saturation (SpO2) ≤ 90% on room air.[7]Till now, the precise determinants of severe COVID-19 are not well defined, but primarily they may be related to host factors rather than viral genetic mutations.[8]

As the number of COVID-19 patients is continually increasing worldwide and the management in ICU has become a major challenge; therefore, early recognition of severe forms of COVID-19 is very necessary for triaging of COVID-19 patients.[9]

The detection of SARS-CoV-2 nucleic acid by real-time reverse-transcription polymerase chain reaction (RT-qPCR) is the gold standard tool of COVID-19 diagnosis and monitoring, but it is time-consuming and has a high percentage of false-negative results.[10,11]Other laboratory tests, such as serum ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin-6 (IL-6), C-reactive protein (CRP), procalcitonin (PCT), creatine kinase (CK), creatinine, cardiac troponin I, D-dimer, lactate dehydrogenase (LDH), whole white blood cells (WBCs) count, neutrophil ratio, lymphocyte count, were assessed as laboratory markers in COVID-19 patients.[12,13]

Therefore, this study aimed to compare the diagnostic and prognostic accuracy of mainly used laboratory biomarkers (CRP, ferritin, IL-6, D-dimer, PCT, and LDH) in sera of severe COVID-19 Egyptian patients, to determine the most appropriate biomarker useful in defining severe COVID- 19 patients.

Subjects and Methods

Ethical statement

The protocol of the current study was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Suez Canal University before starting the study (Ref. no.4953, June 2022). Informed consents were obtained from all the study subjects.

Subjects

The current study included 120 severe COVID-19 patients and 50 apparently healthy subjects (employee and attendants) as controls who were recruited from Suez General Hospital, Egypt.

Methods

Nasopharyngeal swab specimen for SARS-CoV-2 RT-qPCR were obtained using a specific swab (Medical Wire, UK) and then placed in a separate collection tube containing two ml of viral transport medium and immediately sent to the corona virus reference laboratory of Suez Canal University. First, the extraction of the viral RNA was performed using a commercial kit (GeneAll, Seoul, South Korea), according to the manufacturer's instructions. Next, RT-qPCR was performed using LightMix® Modular SARS and Wuhan CoV E-gene kit and using one-step RT-PCR polymerase Mix (Tib-Molbiol, Berlin, Germany), according to the manufacturer's instructions.

Patients with positive SARS-CoV-2 RT-qPCR were subjected to blood sampling for the following tests: Venous blood samples (2ml) were collected from each study subject in EDTA vacutainer tubes for complete blood count (CBC) measurement using an automated blood

counter (Sysmex 5 differential parts, Siemens AG, Erlangen, Germany).

Venous blood samples (5ml) were collected in plain vacutainer tube. Serum was separated and divided onto 2 Eppendorf tubes. Serum samples in one Eppendorf tube was used for measuring ALT, AST, creatinine, urea, LDH, procalcitonin, Iron, Ferritin, D-Dimer levels using a clinical chemistry analyzer (Indiko™ Plus Clinical Chemistry Analyzer, ThermoFisher Scientific), according to the manufacture instructions. CRP was assessed using a Cobas c501 fully automated autoanalyzer (Roche Diagnostics, Manheim, Germany), according to the manufacture instructions.

The other serum sample in the Eppendorf tube was preserved at -20 C for subsequent analysis of serum interleukin (IL)-6 level using an ELISA immunoassay Kits (Cat.NoE0090Hu, Bioassay Technology Laboratory, Shanghai, China), according to the manufacture instructions. Serum detection level in adults ranges from 2-600ng/L.

Chest CT scan were made for all patients compared to normal standard findings. There was no need to do chest CT for controls.

In the current study, severe COVID-19 patients were defined as patients with respiratory distress, resting oxygen saturation ≤ 93%, respiratory failure requiring mechanical ventilation, or failure of other organs requiring ICU admission.

All patients were treated with meropenem (1 gm/8 hrs. intravenous), levofloxacin (500 mg vial/24 hrs., intravenous), Linezolid (600 mg vial / 8 hrs., intravenous), Enoxaparine calcium (therapeutic dose, subcutaneous), methylprednisolone sodium succinate (1 gm every 24 hrs. for 3 days; then 125 mg every 12 hrs.), tocilizumab (if needed, 8 mg/kg), and antiviral.

Severity and management of COVID-19 was graded according to Egypt's Health Ministry announces 7th version of COVID treatment protocol, updates on new medicines; Feb, 2020.[14]

Statistical analysis

Statistical analysis was performed using IBM SPSS software (version 23.0; IBM Corp., Armonk, NY, USA), and data were presented as means ± S.D. One-way ANOVA was used to determine statistically significant differences between group's means and Pearson's correlation coefficient was used to determine significant correlations of serum IL-6 level and other clinical parameters. The Receiver Operating Characteristic curve (ROC curve) was used to calculate the area under the curve (AUC), sensitivity and specificity of CRP, ferritin, IL-6, D-dimer, PCT, and LDH as a biomarker for the detection of severe COVID-19 disease and its bad prognosis. The criterion for significance was $p<0.05$.

Results

Demographic and biochemical data of severe COVID-19 patients

The present study included 120 severe COVID-19 patients; 77 males and 43 females; with mean age of 57.37±9.37 years. The mean oxygen saturation was 85.84±2.05%; respiratory rate 26.83±3.17 cycle/min; and 47 (39.2%) patients died during hospitalization. The clinical and biological data of severe COVID-19 patients and control subjects are summarized in Table 1.

The results of the current study revealed a significant increase in the serum ALT (34.26±18.84) and AST (36.74±16.81) when compared to controls (19.38±7.61& 21.86±9.25, respectively) ($p<0.001$, for both). However, the levels of urea and creatinine were not increased. Whole blood parameters such as WBCs count (12.77±4.48) and neutrophils percentage (74.12±7.01) were significantly increased among severe COVID-19 patients as compared to controls (7.32±1.98 and 64.68±5.01, respectively) ($p<0.001$, for both). However, lymphocytes percentage (19.55±6.36) was significantly decreased when compared to controls (29.94±5.21) ($p<0.001$), as shown in Table1.

Table 1. Clinicopathological characteristics of severe COVID-19 patients and controls.

	Control group (n=50) Mean±SD	Severe COVID-19 Group (n=120) Mean±SD	*p*-value
Gender n (%)			
Male	33 (66.0%)	77 (64.2%)	NS
Female	17 (34.0%)	43 (35.8%)	
Age (years)	54.72±9.19	57.37±9.37	NS
Urea (mg/dl)	37.72±3.96	42.12±6.07	0.032
Creatinine (mg/dl)	1.09±0.01	1.15±0.76	NS
ALT (U/L)	19.38±7.61	34.26±18.84	<0.001
AST (U/L)	21.86±9.25	36.74±16.81	<0.001
WBCs (10^3/µl)	7.32±1.98	12.77±4.48	<0.001
Neutrophils (%)	64.68±5.01	74.12±7.01	<0.001
Lymphocytes (%)	29.94±5.21	19.55±6.36	<0.001
Oxygen saturation (%)	---	85.84±2.05	<0.001
Respiratory rate (cycle/min)	---	26.83±3.17	<0.001
Oxygen supply			
CPAP	---	61 (50.8%)	<0.001
NRM		59 (49.2%)	
Chest CT			
CORADS4	---	44 (36.7%)	<0.001
CORADS5		76 (63.3%)	
Mortality n (%)			
Survivors	50 (100%)	73 (60.8%)	<0.001
Non-survivors	0 (0%)	47 (39.2%)	

CPAP: continuous positive airway pressure; NRM: non-rebreather mask. *P* > 0.05 is not significant (NS).

Levels of serum CRP, ferritin, IL-6, D-dimer, PCT and LDH in severe COVID-19 patients

As depicted in Figures 1A and 1B, the levels of serum CRP (55.97±26.27), ferritin (353.81±177.94), IL-6 (216.58±187.16), D-dimer (0.79±0.32), PCT (0.70±0.28), and LDH (378.78±140.41) were significantly increased in severe COVID-19 patients when compared to the control group (4.95±1.57; 106.68±32.96; 5.25±1.11; 0.05±0.01; 0.13±0.09 and 132.77±15.63, respectively) (p<0.001, for all).

When the severe COVID-19 patients were divided into survivors and non-survivors, our results showed that the levels of serum CRP (61.93±11.94), ferritin (485.53±68.16), IL-6 (408.86±69.75), D-dimer (1.15±0.08), and LDH (476.81±83.26) were significantly increased in non-survivors when compared to survivors (52.12±9.23; 269.01±68.16; 92.79±32.46; 0.56±0.35 and 315.66±24.35, respectively) (p<0.001, for all).

While PCT was significantly increased in non-survivors (0.96±0.08) when compared to survivors (0.53±0.13) (p<0.05), as shown in Figures 1C and 1D.

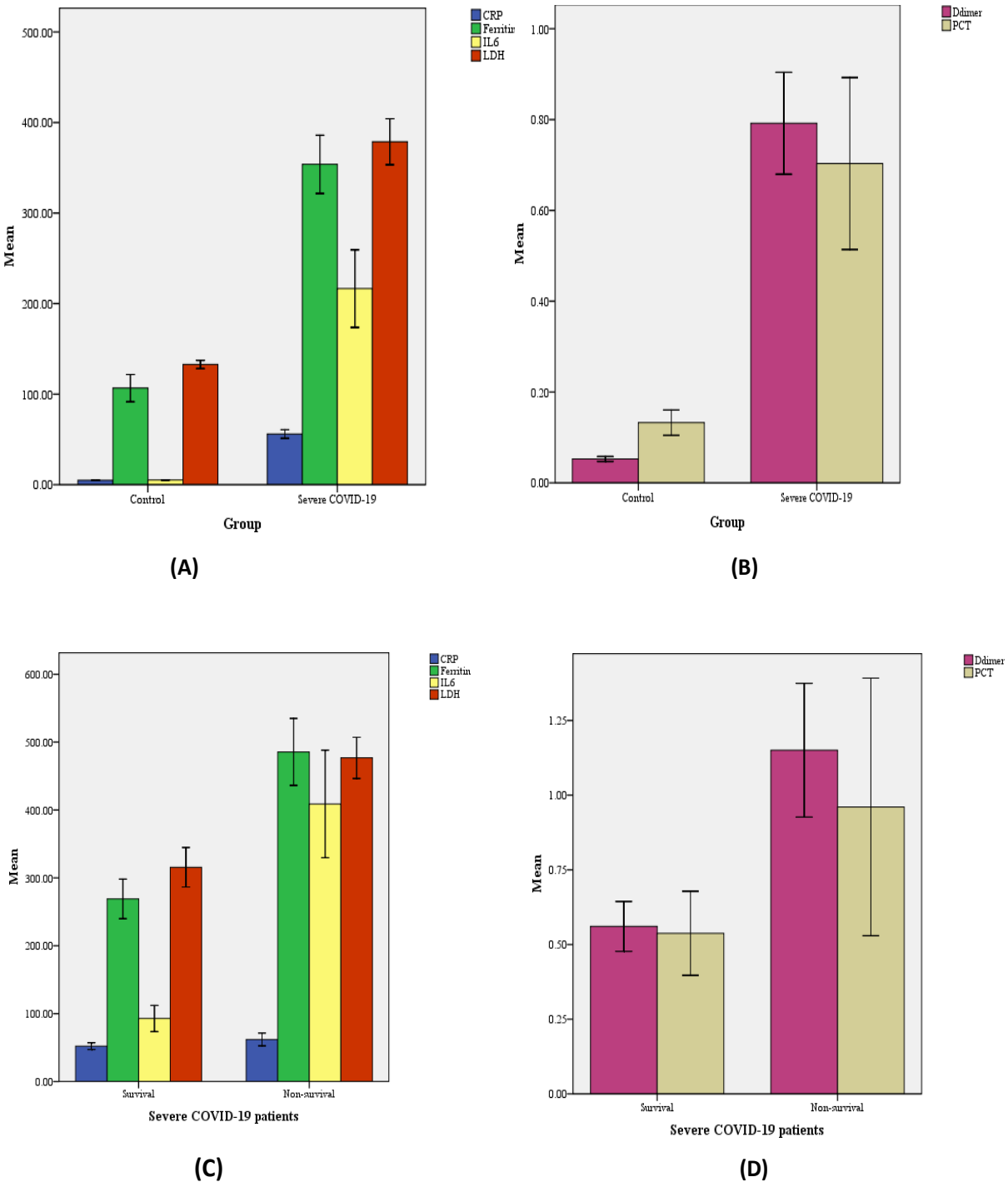


Figure 1. (A) Mean levels of CRP, ferritin, IL-6 and LDH in severe COVID-19 patients and controls; (B) Mean levels of D-dimer and PCT in severe COVID-19 patients and controls; (C) Mean levels of CRP, ferritin, IL-6 and LDH in survival and non-survival COVID-19 patients; and (D) Mean levels of D-dimer and PCT in survival and non-survival COVID-19 patients.

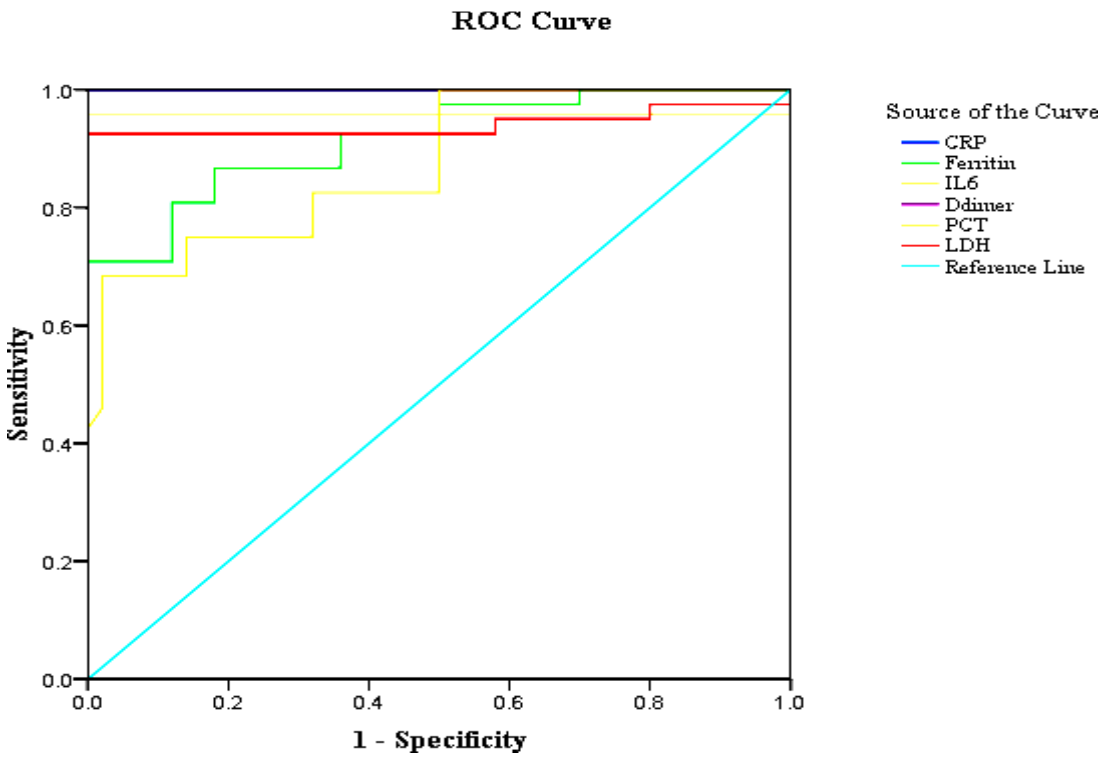
Receiver operating characteristic (ROC) curves analysis

The sensitivity and specificity of CRP, ferritin, IL-6, D-dimer, procalcitonin, and LDH as biomarkers for the diagnosis of severe COVID-19 infection and the prediction of death or bad prognosis of COVID-19 infection were evaluated by using ROC curve analysis. In case of diagnosis of severe COVID-19 infection, our results showed that AUC for CRP was 1.0 with 100% sensitivity and specificity. While AUC for ferritin was 0.914 with 86.7% sensitivity and 82% specificity, AUC for IL-6 0.958 with 95.8% sensitivity and 100% specificity, AUC for D-dimer 1.0 with 90% sensitivity and 100% specificity, AUC for PCT 0.874 with 68% sensitivity and 98% specificity, and AUC for LDH 0.94 with 92.5% sensitivity and 100% specificity, as shown in Table 2 (A) and Figure 2 (A). While for the prediction of death or bad prognosis of COVID-19 infection, AUC for CRP was 0.6 with 61.7% sensitivity and 61.6% specificity, AUC for ferritin 0.85 with 78.7% sensitivity and 80.3% specificity, AUC for IL-6 0.89 with 80.9% sensitivity and 84.9% specificity, AUC for D-dimer 0.8 with 85.1% sensitivity and 79.5% specificity, AUC for PCT 0.65 with 46.8% sensitivity and 69.9% specificity, and AUC for LDH 0.85 with 85.1% sensitivity and 82.8% specificity; as shown in Table 2 (B) and Figure 2 (B).

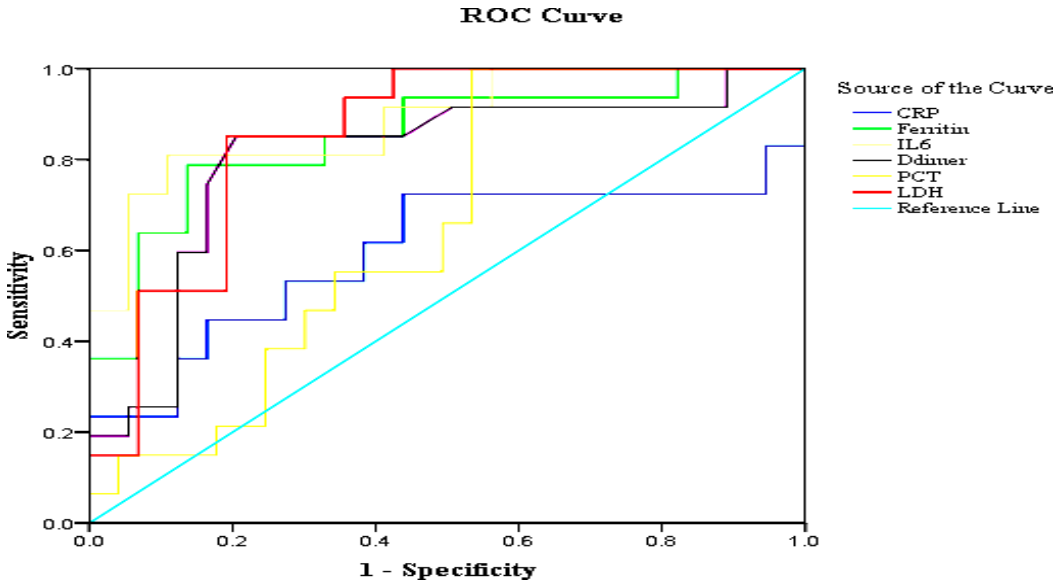
Table 2. Area under the curve (AUC), cut-off value, sensitivity and specificity of CRP, ferritin, IL-6, D-dimer, PCT and LDH in severe COVID-19 patients.

	CPR (mg/dl)	Ferritin (ng/ml)	IL-6 (pg/ml)	D-dimer (mg/L)	PCT (ng/ml)	LDH (U/L)
(A) Diagnosis of severe COVID-19 patients						
AUC	1	0.914	0.958	1	0.874	0.940
Cut-off value	11.05	147.7	7.2	0.33	0.31	177.4
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Sensitivity	100%	86.7%	95.8%	90%	68%	92.5%
Specificity	100%	82%	100%	100%	98%	100%
(B) Prediction of mortality in severe COVID-19 patients						
AUC	0.60	0.85	0.89	0.80	0.65	0.85
Cut-off value	55.5	405.55	170.25	0.71	0.58	405.6
P value	NS	0.0001	0.0001	0.0001	0.004	0.0001
Sensitivity	61.7%	78.7%	80.9%	85.1%	46.8%	85.1%
Specificity	61.6%	80.3%	84.9%	79.5%	69.9%	82.8%

P value for comparison with asymptomatic subjects, $P > 0.05$ is not significant (NS).



(A)



(B)

Figure 2. ROC curve of CRP, ferritin, IL-6, D-dimer, PCT and LDH for: (A) Diagnosis of severe COVID-19 disease; (B) Prediction of mortality in severe COVID-19 patients.

Pearson correlation of serum IL-6 level with other parameters in severe COVID-19 patients

By using Pearson correlation analysis, our results showed that both IL-6 and LDH had the strongest correlations with other parameters among severe COVID-19 patients. Figure 3 A-H showed the correlation of serum IL-6 level with the different studied parameters. It was found that serum IL-6 level had significant positive correlations with CRP (0.621), ferritin (0.526), D-dimer (0.372), PCT (0.467), LDH (0.65) and AST (0.394) (p<0.001, for all). While it had significant positive correlations with WBCs count (0.239) (p<0.05). However, there was a significant negative correlation between serum IL-6 level and lymphocytes percentage (-0.367) (p<0.001).

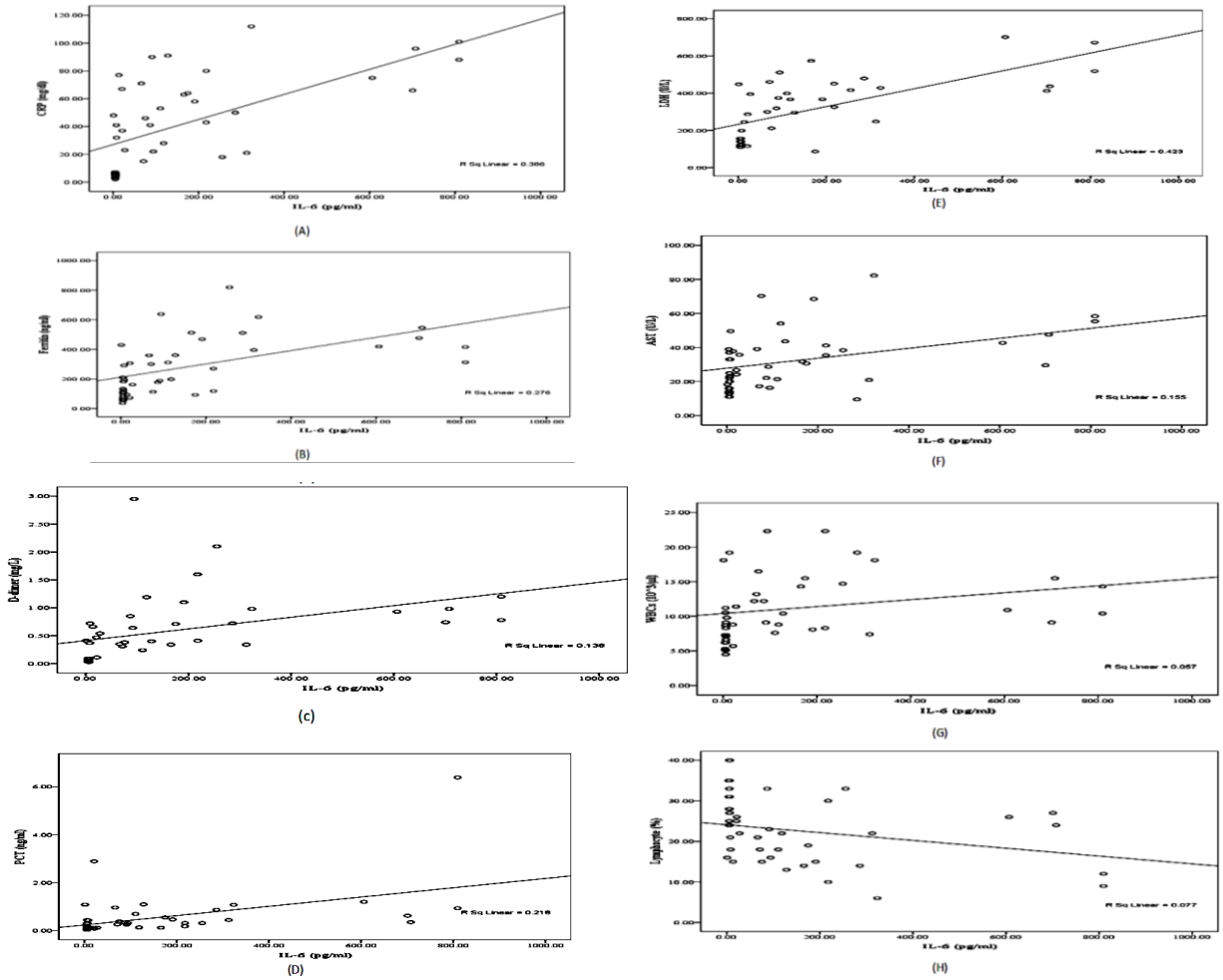


Figure 3. Correlations of serum IL-6 with different parameters among severe COVID-19 patients. CRP (A), ferritin (B), D-dimer (C), PCT (D), LDH (E), AST (F), WBCs (G) and lymphocytes (H).

Figure 4 A-H showed the correlation of serum LDH level with the different parameters in the study. It was found that serum LDH level had significant positive correlations with CRP (0.581), ferritin (0.72), IL-6 (0.65), D-dimer (0.584), PCT (0.375), WBCs count (0.503) and neutrophils percentage (0.4) (p<0.001, for all). However, there was a significant negative correlation between serum LDH level and lymphocytes percentage (-0.478) (p<0.001).

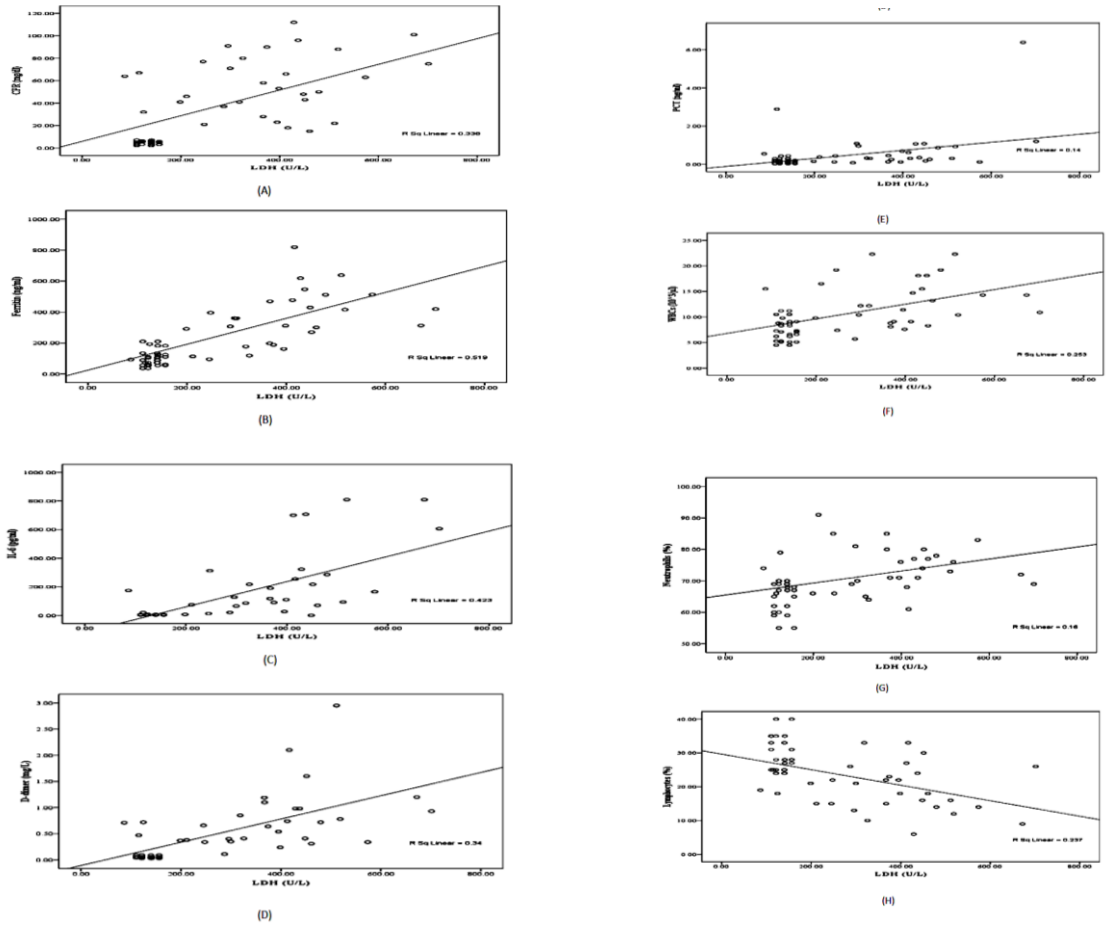


Figure 4. Correlations of serum LDH with different parameters among severe COVID-19 patients. CRP (A), ferritin (B), IL-6 (C), D-dimer (D), PCT (E), WBCs count (F), neutrophils (G) and Lymphocytes (H).

Discussion

In our work, we assessed the diagnostic and prognostic laboratory biomarkers in severe COVID-19 patients admitted to intensive care unit to determine the most appropriate biomarker useful in defining severe COVID-19 patients.

The outbreak of COVID-19 affected about 405 million people causing millions of deaths worldwide. Many countries still suffer from overcrowded hospital wards putting more challenges on health systems despite vaccination and good treatment. Studying the diagnostic and prognostic laboratory biomarkers help the prediction of severely ill patients which in turn may help to reduce morbidity and mortality.

We measured the levels of serum IL-6 and other biomarkers in positive COVID-19 patients to explore their clinical values and significance in discrimination between moderate and severe COVID-19 infection and in predicting the severity and prognosis of COVID- 19 disease. Our results revealed that the serum levels of CRP, ferritin, IL-6, D-dimer, PCT and LDH were significantly increased among severe COVID-19 patients.

Clinical data from Wuhan, China, showed that about 17.7–32.0% of COVID-19 patients need ICU-level care, and the mortality of critically ill patients was 49.0–61.5%.[15, 16]In our study the mortality rate of severe COVID-19 patients is 39.2%.

As consistent with our results, inflammatory markers such as CRP, IL-6, serum ferritin, PCT and LDH were reported to be significantly correlated with the risk of the development of severe COVID-19.[17-20] By using ROC curve

analysis to assess the sensitivity and specificity of these markers, our results showed that CRP was the most sensitive and specific marker for the detection of severe COVID-19 disease, followed by IL-6. While in case of the prediction of death, IL-6 was the most sensitive and specific marker with 80.9% sensitivity and 84.9% specificity. CRP is a non-specific acute-phase pentameric protein, its expression level is significantly increased during acute inflammatory responses.[21, 22] Immunologically, IL-6 and CRP are closely intertwined as IL-6 is known to induce gene expression and production of CRP from hepatocytes and immune cells.[23] Several previous studies reported that IL-6 and CRP were associated with severity of COVID-19 and mortality.[9,19,24]

In our study, IL-6 showed a significant correlation with the other parameters by Pearson correlation analysis, which suggested that IL-6 was a significant factor associated with the severity of patients with COVID-19. A pervious study, reported that in severe COVID-19 patients, the human immune system was hyperactivated and the prominent IL-6 response may suggest that part of the pathogenesis of complicated disease involves a dysregulated and excessive host inflammatory response.[25] The inflammatory response has a crucial role in the pathogenesis of COVID-19 disease, and inflammatory cytokine storm increases the severity of COVID-19.[26] It was reported that cytokine storm is critical to the progression of COVID-19 and can result in severe complications up to even death.[25] A systematic review and meta-analysis reported that IL-6 levels are significantly increased in COVID-19 patients and associated with adverse clinical outcomes. Therefore, inhibition of IL-6 may be a therapeutic target for the management of dysregulated host responses in COVID-19 patients.[25]Another review reported that IL-6 plays a central role in the cytokine storm and elevated in a larger number of severe COVID-19 patients and therefore may be an important cause of death.[27]

According to our results, LDH was the most sensitive and specific marker, second to IL-6, with 85.1% sensitivity and 82.8% specificity in the prediction of death. Also, it has significant correlations with the other parameters by Pearson correlation analysis. LDH is an intracellular enzyme, plays a central role in anaerobic glycolysis as it catalyzes the oxidation of pyruvate to lactate.[28] LDH is an indicator of tissue damage and considered as inflammatory marker. It was significantly increased during acute and severe lung damage, and elevated LDH level was reported in other interstitial lung infections.[29] Since the 1960s, LDH has been used as a traditional marker of cardiac damage. However, abnormal values can result from multiple organ injury, decreased oxygenation and severe infections which cause cytokine-mediated tissue damage.[30] In agreement with our results, several studies reported increased levels of LDH in COVID-19 patients which were associated with disease severity and mortality.[29, 31, 32]

Also consistent with our results, several abnormal hematological parameters such as lymphopenia, neutrophilia, and elevated levels of D-dimer were reported in COVID-19 patients.[33-35] D-dimer is a degradation product of fibrin, produced after activation of coagulation and fibrinolysis.[36] In our study, D-dimer was significantly increased in severe COVID- 19 patients when compared to controls and in non-survivors when compared to survivors. In addition, our results revealed that D-dimer was a good biomarker for diagnosis of severe COVID-19 with 90% sensitivity and 100% specificity; while for death prediction, it had 85.1% sensitivity and 79.5% specificity.

In our study, serum ferritin was significantly increased in non-survivors when compared to survivors, and when used as a biomarker for death prediction; it had 78.7% sensitivity and 80.3% specificity. Previous studies reported that serum ferritin, a feature of hemophagocytic lympho-histiocytosis, is closely related to poor prognosis of COVID-19 patients.[37-39]

Our results revealed that serum PCT level was significantly increased among severe COVID-19 patients, especially in non-survivors, but as a diagnostic or prognostic tool it was a poor biomarker with 68% sensitivity and 98% specificity in severe COVID-19 diagnosis and 46.8% sensitivity and 69.9% specificity in the death prediction between severe COVID-19

patients. PCT is the calcitonin precursor but without hormonal activity. Normally, serum PCT level is very low, while it is increased in the bacterial infections and relatively low in the viral infections. Therefore, it could be used to differentiate between bacterial and viral infections.[40-41] It was reported that PCT level increased in severe COVID-19 patients, which suggesting that severe COVID-19 disease may have concomitant bacterial infection.[19]

In conclusion, our data revealed that serum levels of CRP, ferritin, IL-6, D-dimer, PCT and LDH were significantly increased in severe COVID-19 patients as compared to controls, and in non-survivors as compared to survivors. As biomarkers, CRP and IL-6 may be considered the most appropriate biomarkers in the diagnosis of severe COVID-19 disease, while IL-6 and LDH could possibly be good predictors of mortality between severe COVID-19 patients.

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Author Contributions

MF; deigned the study, collected the samples, selected the participants, interpreted the data and adjusted and revised the paper. SSA; prepared the study design, assembled the data, analyzed and interpreted of the study data and adjusted and revised the paper. SAA, formulated the idea and design, put the study plan, wrote the study protocol for having Suez Canal University ethics committee approval, analyzed the samples, interpreted the data, edited and revised the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the current study was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Suez Canal University before starting the study (Ref. no.4953, June 2022).

Informed consent

Informed consents were obtained from all the study subjects.

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