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Interpretation of Hematological, Biochemical, and Immunological Findings of COVID-19 Disease: Biomarkers Associated with Severity and Mortality

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Biomarkers Associated with Severity and Mortality of COVID-19

ABSTRACT

The severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) spread rapidly all over the world in late 2019 and caused critical illness and death in some infected patients. This study aimed at examining several laboratory factors, especially inflammatory and immunological mediators, to identify severity and mortality associated biomarkers.

Ninety-three hospitalized patients with confirmed coronavirus disease 2019 (COVID-19) were classified based on disease severity. The levels of biochemical, hematological, immunological, and inflammatory mediators were assessed, and their association with severity and mortality were evaluated.

Hospitalized patients were mostly men (77.4%) with an average (standard deviation) age of 59.14 (14.81) years. The mortality rate was significantly higher in critical patients (85.7%). Increased serum levels of blood sugar, urea, creatinine, uric acid, phosphorus, total bilirubin, serum glutamic-oxaloacetic transaminase, serum glutamic-oxaloacetic transaminase, lactic dehydrogenase, C-reactive protein, ferritin, and procalcitonin were significantly prevalent (p=0.002, p<0.001, p<0.001, p=0.014, p=0.047, p=0.003, p<0.001, p<0.001, p<0.001, p<0.001, P<0.001, and p<0.001, respectively) in COVID-19 patients. Decreased red blood cell, hemoglobin, and hematocrit were significantly prevalent among COVID-19 patients than healthy control subjects (p<0.001 for all). Troponin-I, interleukin-6, neutrophil/lymphocyte ratio (NLR), procalcitonin, and D-dimer showed a significant association with the mortality of patients with specificity and sensitivity more than 60%.

Age, sex, underlying diseases, blood oxygen pressure, complete blood count along with C-reactive protein, lactic dehydrogenase, procalcitonin, D-dimer, and interleukin-6 evaluation help to predict the severity and required management for COVID-19 patients. Further investigations are highly recommended in a larger cohort study for validation of the present findings.

Keywords: Biomarkers; COVID-19; Immunology; Inflammation; SARS-CoV-2

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with a zoonotic origin; appeared in late 2019 and caused the coronavirus disease 2019 (COVID-19).[1-3]

A pandemic state in less than three months by the World Health Organization (WHO) after the rapid spread of the disease with its significant mortality highlighted urgent studies on this topic. SARS-CoV-2 affects several organs, including the lungs, kidneys, and liver. It may also result in intravascular coagulation and central nervous system problems.[4] However, SARS-CoV-2 mainly affects the lower respiratory tract resulting in atypical pneumonia. This involvement may result in severe complications since the pathogen causes acute respiratory distress syndrome (ARDS) with the urgent need for particular management at intensive care units (ICUs).[5] ARDS and mortality of COVID-19 patients are associated with the dysregulation of immunological and inflammatory responses.[6] Cytokine release syndrome (CRS) is the underlying factor for the induction of ARDS, and its link with the morbidity of COVID-19 patients has been documented. Moreover, several studies have reported various immune-related cellular and molecular changes in these patients. The most significant changes are lymphopenia, neutrophilia, uncontrolled increase in inflammatory cytokines (cytokine storm), especially interleukin (IL) -6, tumor necrosis factor (TNF) -α, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1 (MIP1), and other inflammation-related factors, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, albumin, and transferrin, and also increased coagulation factors such as D-dimer.[7-10]

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Various studies have shown the association of lymphopenia, hyper inflammation, and coagulation with the pathogenesis of COVID-19. The data on the contributing risk factors in the pathogenesis of COVID-19 is limited. This study aimed at investigating the clinical and paraclinical parameters of Iranian COVID-19 patients hospitalized in Tehran City, Iran, since these data may play a crucial role in identifying the correlation of biomarkers with the severity and mortality of the disease. Moreover, the relationship between different parameters may help the management and follow up of the COVID-19 patients.

MATERIALS AND METHODS

Study Population and Ethical Considerations

A total of 125 consecutive inpatients suspected of COVID-19 hospitalized in Tehran hospitals were enrolled in our study (from February 12 to April 4, 2020). Besides, we used the cluster sampling method to recruit 67 SARS-CoV-2 RT-PCR (real-time polymerase chain reaction), negative clinically-proven healthy volunteers, as the healthy control (HC) group. The diagnosis was made based on the World Health Organization interim guidance.[11] In this regard, nasopharyngeal swabs for SARS-CoV-2 RT-PCR and chest computed tomography (CT) scans were performed for enrolled subjects. Thirty-two COVID-19 suspected participants were excluded because of negative SARS-CoV-2 RT-PCR and major interfering complications such as malignancy and pregnancy. Two clinicians were independently collected demographic data, significant clinical procedures, clinical characteristics, radiological findings, and outcomes to increase the accuracy and precision of data collected. A final follow-up in May 2020 was performed to record the outcome of all patients.

The severity of the disease is classified into three subgroups based on the types of oxygen therapies. Patients with supportive O_2 nasal cannula or mask are considered as the moderate group. Those admitted to the intensive care unit (ICU) who received non-invasive ventilation (NIV) masks were categorized as the severe group. Subjects admitted to ICU and used mechanical ventilator (intubated) were considered as the critical patients or group.

The study was approved by the National Ethics Committee on Research in Medical Sciences of the Iranian Ministry of Health (IR.NIMAD.REC.1398.411), and written informed consent was obtained from all participants.

Sample Preparation

Peripheral blood samples were obtained in the ethylenediaminetetraacetic acid (EDTA) treated Vacutest and Gel, and Clot activator tubes (Kima, Italy) for hematology assays, serum, and plasma preparation, respectively. Separation and preparation of the whole blood specimens were conducted under a safe procedure. Sera were isolated after coagulation and centrifuged at 3000 rpm for 15 min at room temperature and then used freshly for biochemistry and immunoassays. Furthermore, all samples were kept frozen at -80ºC for assessing cytokines and other relevant factors.

Hematological and Biochemical Assays

Complete blood count (CBC) was performed using Automated Sysmex (XS 500i full diff, Japan). Also, we used Hitachi-91 auto-analyzer (Japan) to measure blood sugar (117500, Pars Azmun, Iran), urea (DDP01193-L, Delta. DP, Iran), creatinine (109400, Pars Azmun, Iran), uric acid (130400, Pars Azmun, Iran), triglyceride (DDP01192-L, Delta. DP, Iran), phosphorus (DDP0118-S, Delta. DP, Iran), total bilirubin (5020, Pars Azmun, Iran), serum glutamic oxaloacetic transaminase (SGOT) (DDP01159-L, Delta. DP, Iran), serum glutamic pyruvic transaminase (SGPT) (DDP01154-L, Delta. DP, Iran), alkaline phosphatase (ALP) (1400, Pars Azmun, Iran), creatine phosphokinase (CPK) (DDP01166-S, Delta. DP, Iran), lactate dehydrogenase (LDH) (DDP01182-S, Delta. DP, Iran), and C-reactive protein (CRP) (3040, BIONIK DIAGNOSTIC SYSTEMS, Iran). The serum levels of procalcitonin (PCT) (VIDAS PCT) and troponin I (VIDAS TNHS) were measured using VIDAS bioMerieux (France), and serum levels of D-dimer (L2KDD2), and ferritin (L2KFE2) were analyzed using an automated immunoassay (IMMULITE 2000, Siemens Healthineers, the United Kingdom).

Cytokines and Complement Factors Measurement

Tumor necrosis factor-alpha (TNFα), interleukin-1-beta (IL-1-β), interleukin-1 receptor antagonist (IL-1Ra), IL-8, and IL-10 were measured in serum samples using DouSet ELISA Development System (all from R&D Systems, catalog number: DY210, DY201,

DY280, DY217B, respectively). Serum levels of IL-6 (L2K6P2) were assessed using an automated immunoassay (IMMULITE 2000 Immunoassay System, Siemens Healthcare Diagnostics Inc., The United States of America).

Statistical Analysis

The statistical analyses were done using SPSS (version 24.0, IBM SPSS Co, Armonk, NY). Demographic information, vital signs on admission, and time from the onset of the disease to hospitalization were reported as mean±standard deviation (SD) and compared between groups using Welch corrected t-test and Tukey post hock pairwise comparison. Symptoms, comorbidity, and other qualitative factors were compared using the Chi-square test. Para-clinical findings were reported as mean±SD or median and compared using the Mann-Whitney *U* test or t-test. The correlation of para-clinical parameters with each other and mortality was computed using the Spearman rank correlation coefficient. The area under the receiver operating curve (AUC) was calculated for some of the para-clinical factors. The best cut-off point was set as a point with maximum sensitivity and specificity. A *p*-value of less than 0.05 was considered significant.

RESULTS

Increased Mortality of Critical COVID-19 Patients

The basic information of the study groups is presented in Table 1. The study sample comprised 72 males (77.4%) and 21 females (22.6%) confirmed hospitalized patients with SARS-CoV-2. Fifty-five males and 13 females were added to the healthy control group. The gender proportion was not significantly different between COVID-19 patients and HC groups (P = 0.595). . Based on the disease severity, the patients were subdivided into three groups as explained earlier. Forty-three patients (46.2%) in the moderate, 15 patients (16.1%) in the severe, and 21 patients (22.6%) were in the critical group. Respiratory support data was missing for fourteen patients. These patients could not be classified. However, their laboratory data were used in the comparison of all COVID-19 patients with HC. The mean±SD age of COVID-19 patients was higher than that in the control subjects (59.14 ± 14.81 vs. 52.78±11.77, p=0.004). To remove the probable covariance effect of age, analysis of covariance was performed and it was demonstrated that the age did not have a covariance effect on the results (data was not shown). As presented in Table 1, there was no significant difference between the age and the gender proportion of the three subgroups of COVID-19 patients. All patients in the moderate and severe groups were eventually discharged in contrast to the critical group which had an 85.7% mortality rate (18 of 21 patients).

Decreased oxygen saturation (SpO_2) on admission were significantly prevalent among the critical as compared to moderate patients (p=0.030) (Table 1). Additionally, increased systolic blood pressure (SBP) on admission were prevalent in the critical group in comparison to moderate patients (p=0.041) (Table 1). Moreover, of all symptoms, only chest pain was reported to be significantly common among severe as compared to the critical patients (p=0.032). Almost all of the included critical and severe patients had at least one of the above-mentioned comorbidities, which were significantly prevalent among COVID-19 patients compared to HC (p<0.001). It is noteworthy to mention that because of the small sample size, most of the comorbidities created no statistically significant difference between the study groups (Table 1).

Systemic corticosteroids, antibiotics, atazanavir, intravenous immunoglobulin (IVIG), interferon-beta (IFN-β), and vitamin-C prescription were more prevalent for severe patients as compared to moderate ones (p<0.001, p=0.012, p<0.001, p=0.004, p=0.018, and p=0.004, respectively) (Table 1). Additionally, critical patients were prescribed systemic corticosteroids, antibiotics, atazanavir, sofosbuvir, IVIG, and IFN-β prescription as compared to moderate patients (p=0.005, p=0.002, p=0.002, p=0.007, p<0.001, and p=0.023, respectively) (Table 1). Moreover, only ribavirin was prescribed significantly more in critical patients compared to severe patients (p=0.042) (Table 1).

Dysregulation of Biochemical and Hematological Findings with Disease Severity

Increased serum levels of blood sugar, urea, creatinine, uric acid, phosphorus, total bilirubin, SGOT, SGPT, LDH, CRP, ferritin and PCT were significantly prevalent (p=0.002, p<0.001, p<0.001, p=0.014, p=0.047, p=0.003, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001 and p<0.001, respectively) in COVID-19 patients compared to the HC (Table 2). Of note, mean (SD) values of serum CRP, ferritin, and

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Table 1. The basic and clinical information of COVID-19 patients based on the disease severity

		Moderate (n=43)	Severe (n=15)	Critical (n=21)	*p* 1	*p* 2	*p* 3
Age (y)	< 55	14 (33.3%)	4 (26.7%)	5 (23.8%)	0.633	0.437	0.845
	≥ 55	28 (66.7%)	11 (73.3%)	16 (76.2%)			
Gender (male/female)		37/6 (86%)	10/5 (66.7%)	14/7 (66.7%)	0.099	0.070	>0.999
Time from onset to Hospitalization (d)		6.62 (± 4.30)	6.73 (± 3.17)	7.07 (± 3.65)	0.853	0.728	0.916
Outcome (deceased)		0/43 (0.0%)	0/15 (0.0%)	18/21 (85.7%)	-	**<0.001***	**<0.001***
Vital signs on admission							
	SpO_2 (%)	90.49 (± 4.64)	88.73 (± 5.02)	86.86 (± 5.64)	0.335	**0.030***	0.215
	SBP (mm Hg)	117.82 (±10.07)	119.85 (± 15.50)	130.86 (± 24.28)	0.604	**0.041***	0.196
	DBP (mm Hg)	77.46 (± 6.47)	78.23 (± 7.64)	74.83 (± 10.31)	0.569	0.399	0.339
	T (°C)	37.46 (± 0.90)	37.51 (± 1.23)	37.56 (± 0.85)	0.791	0.549	0.539
	PR (beats/min)	97.40 (± 15.92)	92.33 (± 13.55)	99.20 (± 18.95)	0.173	0.947	0.361
	RR (beats/min)	21.73 (± 5.35)	23.27 (± 6.25)	21.15 (± 5.15)	0.343	0.568	0.244
Symptoms							
	Fever	28/42 (66.7%)	11/15 (73.3%)	14/21 (66.7%)	0.633	>0.999	0.669
	Dry cough	30/42 (71.4%)	12/15 (80%)	11/21 (52.4%)	0.518	0.135	0.089
	Dyspnea	30/42 (71.4%)	9/15 (60%)	16/21 (76.2%)	0.414	0.688	0.298
	Myalgia	27/42 (64.3%)	11/15 (73.3%)	12/21 (57.1%)	0.523	0.582	0.319
	Chest pain	5/42 (11.9%)	3/15 (20%)	0/21 (0%)	0.438	0.099	**0.032***
	Fatigue	15/42 (35.7%)	6/15 (40%)	3/21 (14.3%)	0.768	0.076	0.079
	Headache	5/42 (11.9%)	1/15 (6.7%)	2/21 (9.5%)	0.570	0.777	0.760

	Sore throat	5/42 (11.9%)	1/15 (6.7%)	1/21 (4.8%)	0.570	0.363	0.806
	GI related	17/42 (40.5%)	7/15 (46.7%)	4/21 (19%)	0.677	0.089	0.076
	Hemoptysis	3/42 (7.1%)	0/15 (0%)	1/21 (4.8%)	0.288	0.715	0.391
	Sputum production	0/42 (0%)	1/15 (6.7%)	0/21 (0%)	0.091	-	0.230
	Rhinorrhea	1/42 (2.4%)	0/15 (0%)	1/21 (4.8%)	0.547	0.611	0.391
Comorbidities		26/42 (57.8%)	11/15 (61.1%)	19/21 (73.1%)	0.808	0.197	0.402
	More than one comorbidity	13/42 (28.9%)	8/15 (44.4%)	12/21 (46.2%)	0.237	0.142	0.911
	Diabetes mellitus	11/42 (26.2%)	6/15 (40%)	8/21 (38.1%)	0.316	0.332	0.908
	Hypertension	17/42 (40.5%)	8/15 (53.3%)	7/21 (33.3%)	0.389	0.582	0.230
	Cardiovascular disease	7/42 (16.7%)	5/15 (33.3%)	6/21 (28.6%)	0.174	0.271	0.760
	Chronic kidney disease	5/42 (11.9%)	1/15 (6.7%)	4/21 (19%)	0.570	0.445	0.290
	Respiratory diseases	4/42 (9.5%)	1/15 (6.7%)	3/21 (14.3%)	0.737	0.571	0.473
	Cerebrovascular complications	1/42 (2.4%)	0/15 (0%)	2/21 (9.5%)	0.547	0.209	0.219
	Cancer	0/42 (0%)	1/15 (6.7%)	2/21 (9.5%)	0.091	**0.042***	0.760
	Immune system disorder	0/42 (0%)	1/15 (6.7%)	1/21 (4.8%)	0.091	0.154	0.806
	Thyroid disorder	0/42 (0%)	2/15 (13.3%)	3/21 (14.3%)	**0.016***	**0.012***	0.935
Treatment							
	Systemic corticosteroids	9/42 (21.4%)	11/15 (73.3%)	12/21 (57.1%)	**< 0.001***	**0.005***	0.319
	Antibiotics	15/42 (35.7%)	11/15 (73.3%)	16/21 (76.2%)	**0.012***	**0.002***	0.845
	Hydroxychloroquine	39/42 (92.9%)	14/15 (93.3%)	16/21 (76.2%)	0.951	0.061	0.174
	Oseltamivir	35/42 (83.3%)	10/15 (66.7%)	16/21 (76.2%)	0.174	0.496	0.529
	Lopinavir/ ritonavir	27/42 (64.3%)	6/15 (40%)	9/21 (42.9%)	0.102	0.105	0.864

Atazanavir	11/42 (26.2%)	12/15 (80%)	14/21 (66.7%)	**< 0.001***	**0.002***	0.379
Sofosbuvir	2/42 (4.8%)	2/15 (13.3%)	6/21 (28.6%)	0.265	**0.007***	0.278
Ribavirin	3/42 (7.1%)	0/15 (0%)	5/21 (23.8%)	0.288	0.061	**0.042**
IVIG	2/42 (4.8%)	2/42 (4.8%)	9/21 (42.9%)	**0.004***	**< 0.001***	0.563
IFN-β	2/42 (4.8%)	2/42 (4.8%)	5/21 (23.8%)	**0.018***	**0.023***	0.845
Vitamin C	1/42 (2.4%)	4/15 (26.7%)	2/21 (9.5%)	**0.004***	0.209	0.174
Vitamin D	7/42 (16.7%)	3/15 (20%)	1/21 (4.8%)	0.771	0.181	0.151

Data are presented as mean (±standard deviation), n (%), or n/N (%), where N is the total number of patients with available data. Statistical analysis was performed using t-test and χ^2 test.

*p*1 (*p*-value 1), comparison between patients with moderate and severe complications; *p*2 (*p*-value 2), comparison between patients with moderate and critical complications; *p*3 (*p*-value 3), comparison between patients with severe and critical complications; * $p<0.05$ was regarded as statistically significant.

SpO_2, oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; T, temperature; PR, pulse rate; HR, heart rate; RR, respiratory rate, IVIG; intravenous immunoglobulin, IFN-β; interferon-beta, GI; Gastrointestinal.

PCT are significantly high in COVID-19 compared to HC (Figure 1). Besides, increased levels of serum troponin I and D-dimer were reported in critical as compared to moderate COVID-19 subjects (p=0.013 and <0.001, respectively) (Table 2). Mean (SD) serum D-dimer levels significantly elevates with the disease severity (Figure 1).

As shown in Table 2, the amount of leukocytosis (WBC≥11000 per microliter) and leukopenia (WBC≤4100 per microliter) in patients with COVID-19 significantly increased compared to those in HCs (p<0.001 and p=0.003, respectively). Leukocytosis and leukopenia were significantly more prevalent in critical and moderate COVID-19, respectively (p= 0.006 and p=0.029, respectively) (Table 2). In this regard, the mean (SD) value of WBC significantly decreased in the moderate group (p=0.008) and increased in severe and critical COVID-19 patients (p=0.011 and p=0.001, respectively) as compared to the HC group (Figure 1). Neutrophilia (neutrophil≥6300 per microliter) was also significantly prevalent in COVID-19 patients (p<0.001), especially in severe and critical patients (p<0.001 for both) (Table 2). In contrast, lymphopenia (Lymphocyte≤1000 per microliter) was significantly prevalent in COVID-19 patients as compared to the HC group (p<0.001) (Table 2). Eventually, increased neutrophil to lymphocyte ratio (NLR) was significantly more prevalent in COVID-19 patients (p<0.001), and this condition was more pronounced in severe and critical patients as compared to the moderate groups (p=0.016 and p=0.004, respectively) (Table 2). Almost similarly, the increased mean (SD) value of lymphocyte count and decreased mean (SD) of neutrophil count and N/L ratio were reported as the severity of the disease increased (Figure 1). Finally, reduced count of eosinophils was significantly more prevalent among COVID-19 patients as compared to HC*s* (p<0.001), which is consistent with the decreased mean (SD) count of eosinophils in all groups of COVID-19 patients as compared to the HC group (p<0.001 for all) (Figure 1).

Decreased RBC, Hb, and HCT were significantly prevalent among COVID-19 patients as compared to the HC group (p<0.001 for all) (Table 2). The altered level of platelets was also significantly prevalent among COVID-19 patients as compared to the HC group (p=0.004 and p=0.015, respectively) (Table 2).

IL-6, IL-8, and IL-1Ra as Hallmarks of COVID-19

The median of serum TNF-α and IL-1β decreased in moderate COVID-19 as compared to the HC patients (p=0.018 and p=0.002, respectively) (Figure 1).

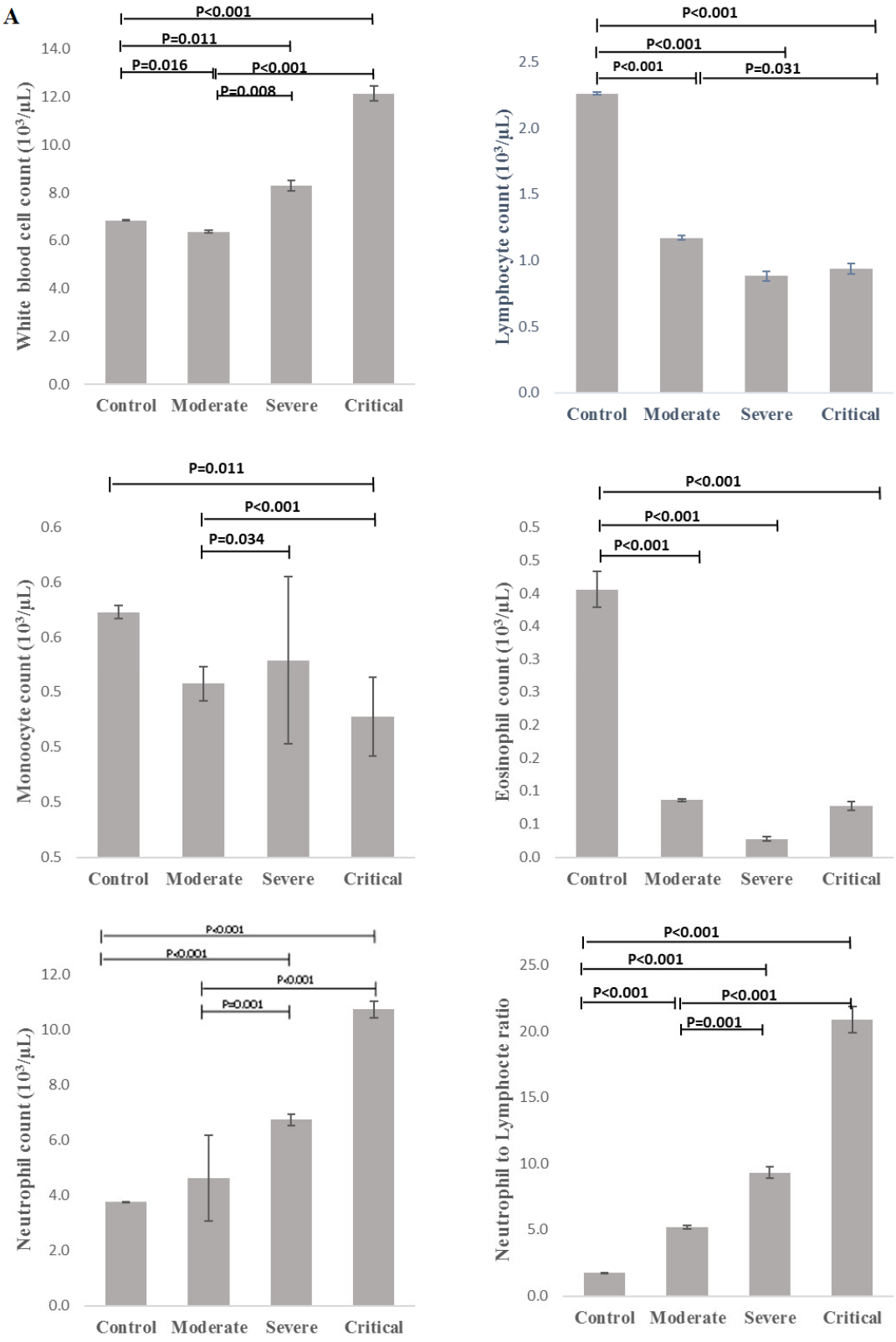
Table 2. The laboratory findings of COVID-19 patients based on the disease severity

	Cut-off	HC	COVID-19	*p*1	Moderate	Severe	Critical	*p*2	*p*3	*p*4
Blood sugar (mg/dL)	≥115	12/66 (18%)	22/49 (45%)	**0.002***	9/23 (39%)	4/6 (67%)	5/9 (56%)	0.227	0.400	0.667
Urea (mg/dL)	≥45	0/66 (0%)	33/70 (47%)	**<0.001***	12/30 (40%)	5/12 (42%)	7/14 (50%)	0.921	0.533	0.671
Creatinine (mg/dL)	≥1.4	0/67 (0%)	11/64 (17%)	**<0.001***	6/30 (20%)	1/7 (14%)	2/13 (15%)	0.728	0.721	0.948
Uric Acid (mg/dL)	≥7.2	1/67 (1%)	6/47 (13%)	**0.014***	3/21 (14%)	1/6 (17%)	1/10 (10%)	0.885	0.739	0.696
Triglycerides (mg/dL)	≥200	12/58 (21%)	13/46 (28%)	0.369	5/21 (24%)	3/6 (50%)	3/9 (33%)	0.215	0.589	0.519
Phosphorus (mg/dL)	≥4.5	1/67 (1%)	5/53 (9%)	**0.047***	0/26 (0%)	2/6 (33%)	1/10 (10%)	**0.002***	0.102	0.247
	≤2.6	3/67 (4%)	13/53 (25%)	**0.001***	8/26 (31%)	1/6 (17%)	4/10 (40%)	0.489	0.599	0.330
Total Bilirubin (mg/dL)	≥1.2	15/67 (22%)	24/49 (49%)	**0.003***	9/23 (39%)	5/5 (100%)	4/10 (40%)	**0.014***	0.963	**0.025***
SGOT (U/L)	≥37	5/67 (7%)	32/69 (46%)	**<0.001***	15/35 (43%)	2/7 (29%)	7/13 (54%)	0.482	0.497	0.279
SGPT (U/L)	≥41	8/67 (12%)	29/68 (43%)	**<0.001***	16/34 (47%)	3/7 (43%)	5/13 (38%)	0.839	0.596	0.848
ALP (U/L)	≥306	2/67 (3%)	6/68 (9%)	0.151	3/34 (9%)	1/7 (14%)	1/13 (8%)	0.657	0.901	0.639
CPK (mcg/L)	≥190	7/64 (11%)	9/68 (13%)	0.686	3/29 (10%)	0/0 (0%)	2/12 (17%)	0.247	0.574	0.140
LDH (U/L)	≥480	2/66 (3%)	64/78 (82.1%)	**<0.001***	29/37 (78.4%)	8/12 (66.7%)	14/15 (93.3%)	0.412	0.197	**0.076**
CRP (U/L)	≥6	7/67 (10%)	71/74 (96%)	**<0.001***	31/32 (97%)	12/12 (100%)	13/14 (93%)	0.536	0.539	0.345
Ferritin (ng/mL)	≥365	1/68 (1%)	69/93 (74%)	**<0.001***	33/42 (79%)	10/15 (67%)	15/20 (75%)	0.358	0.753	0.589
	≥1500	0/68 (0%)	14/93 (15%)	**0.001***	4/42 (10%)	2/15 (13%)	4/20 (20%)	0.680	0.250	0.605
Procalcitonin (ng/mL)	≥0.5	0/68 (0%)	11/58 (19%)	**<0.001***	2/25 (8%)	0/7 (0%)	5/15 (33%)	0.440	**0.041***	0.082
Troponin I (ng/mL)	≥38	-	6/42 (14%)	-	1/21 (5%)	0/9 (0%)	4/10 (40%)	0.699	**0.013***	0.188
	≤15	-	46/57 (81%)	-	28/29 (97%)	8/9 (89%)	4/11 (36%)	0.368	**<0.001***	**0.017**
D-Dimer (ng/mL)	≥15000	-	10/77 (13%)	-	0/37 (0%)	1/9 (11%)	6/18 (33%)	**0.040***	**<0.001***	0.214
	≥10000	-	41/77 (53%)	-	13/37 (35%)	6/9 (67%)	15/18 (83%)	0.085	**0.001***	0.326
WBC ($10^3/\mu L$)	≥11	1/67 (1%)	22/87 (25%)	**<0.001***	5/39 (13%)	3/12 (25%)	9/20 (45%)	0.310	**0.006***	0.258

	≤4.1	0/67 (0%)	11/87 (13%)	**0.003***	8/39 (21%)	1/12 (8%)	0/20 (0%)	0.333	**0.029***	0.190
Neutrophils (10^3/μL)	≥6.3	1/67 (1.5%)	37/87 (42.5%)	**<0.001***	6/40 (15%)	9/12 (75%)	14/21 (66.7%)	**<0.001***	**<0.001***	0.616
Lymphocyte (10^3/μL)	≤1	1/67 (1.5%)	53/87 (60.9%)	**<0.001***	18/40 (45%)	8/12 (66.7%)	14/21 (66.7%)	0.188	0.107	> 0.999
NLR	≥3.53	1/67 (1.5%)	61/87 (70.1%)	**<0.001***	19/40 (47.5%)	12/12 (100%)	18/21 (85.7%)	**0.016***	**0.004***	0.170
Monocyte (10^3/μL)	> 0.5	25/67 (37.3%)	42/87 (48.3%)	0.174	19/40 (47.5%)	6/12 (50%)	11/21 (52.4%)	0.879	0.717	0.895
Eosinophil (10^3/μL)	≤0.01	0/67 (0 %)	39/87 (44.8%)	**<0.001**	9/21 (42.9%)	8/12 (66.7%)	14/40 (35%)	**0.051**	0.547	0.188
RBC (10^6/μL)	≥5.3	35/67 (52%)	13/87 (15%)	**<0.001***	6/39 (15%)	2/12 (17%)	3/20 (15%)	0.915	0.969	0.900
	≤4.3	1/67 (1%)	43/87 (49%)	**<0.001***	17/39 (44%)	7/12 (58%)	11/20 (55%)	0.371	0.406	0.854
Hb (10^3/μL)	≤13.5	8/67 (12%)	63/87 (72%)	**<0.001***	26/39 (67%)	10/12 (83%)	15/20 (75%)	0.268	0.511	0.581
HCT (10^3/μL)	≥45	30/67 (45%)	8/87 (9%)	**<0.001***	4/39 (10%)	1/12 (8%)	1/20 (5%)	0.845	0.493	0.706
	≤38	1/67 (1%)	50/87 (57%)	**<0.001***	22/39 (56%)	10/12 (83%)	10/20 (50%)	0.092	0.640	0.059
Platelets (10^3/μL)	≥450	0/67 (0%)	10/87 (11%)	**0.004***	7/39 (18%)	1/12 (8%)	2/20 (10%)	0.423	0.421	0.876
	≤150	3/67 (4%)	15/87 (17%)	**0.015***	4/39 (10%)	3/12 (25%)	6/20 (30%)	0.194	0.056	0.761
TNF-α (ng/L)	≥8.1	10/54 (19%)	15/64 (23%)	0.515	5/31 (16%)	2/7 (29%)	5/15 (33%)	0.433	0.185	0.823
IL-1 β (ng/L)	≥5	2/59 (3%)	1/64 (2%)	0.512	0/31 (0%)	0/7 (0%)	1/15 (7%)	-	0.146	0.484
IL-6 (ng/L)	>6	8 (11.6%)	93 (84.5%)	**<0.001***	38 (84.4%)	13 (72.2%)	23 (88.5%)	0.170	0.639	0.264
IL-1 Ra (ng/L)	≥1	4/59 (6.8%)	16/63 (25.4%)	**0.006***	4/32 (12.5%)	1/7 (14.3%)	7/15 (46.7%)	0.898	**0.010***	0.141
IL-10 (ng/L)	≥9.1	9/59 (15%)	10/64 (16%)	0.955	1/31 (3%)	2/7 (29%)	4/15 (27%)	**0.025***	**0.017***	0.926
IL-8 (ng/L)	≥62	3/63 (5%)	11/57 (19%)	**0.013***	3/27 (11%)	1/7 (14%)	5/15 (33%)	0.816	0.079	0.350

Data are presented as n/N (%), where N is the total number of patients with available data. Statistical analysis was performed using the χ^2 test. *p*1 (*p*-value 1), comparison between the healthy control and COVID-19 patients; *p*2 (*p*-value 2), comparison between the patients with moderate and severe complications; *p*3 (*p* value 3), comparison between the patients with moderate and critical complications; *p*4 (*p*-value 4), comparison between patients with severe and critical complications; * $p<0.05$ was regarded as statistically significant.

Abbreviations: COVID-19, coronavirus disease of 2019; HC, healthy control group; LDH, lactate dehydrogenase; SGOT, serum glutamic oxaloacetic transaminase or aspartate aminotransferase (AST); SGPT, serum glutamic pyruvic transaminase or alanine aminotransferase (ALT); ALP, alkaline phosphatase; CPK creatine phosphokinase; CRP, c-reactive protein, WBC, white blood cell; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; NLR, neutrophil-lymphocyte ratio; IL, interleukin; TNF-α, tumor necrosis factor-alpha; IL-1Ra, interleukin-1 receptor antagonist; mg/dL, milligrams per deciliter; ng/mL, nanograms per milliliter; μg/L, micrograms per liter; U/L, unit per liter.



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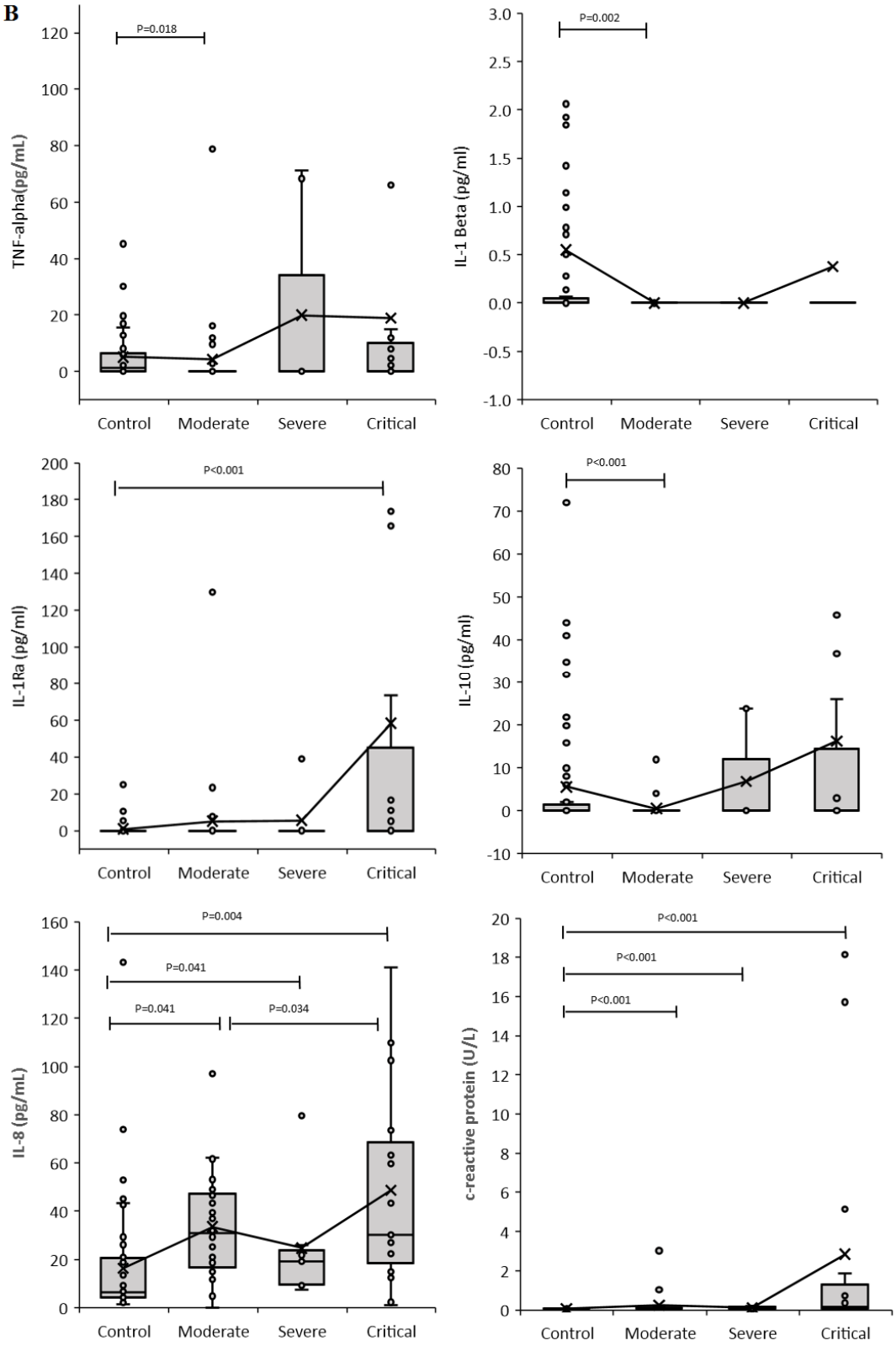
B
TNF-alpha(pg/mL)
P=0.018
IL-1 Beta (pg/ml)
P=0.002
IL-1Ra (pg/ml)
P<0.001
IL-10 (pg/ml)
P<0.001
IL-8 (pg/mL)
P=0.004
P=0.041
P=0.041
P=0.034
c-reactive protein (U/L)
P<0.001
P<0.001
P<0.001
Control
Moderate
Severe
Critical

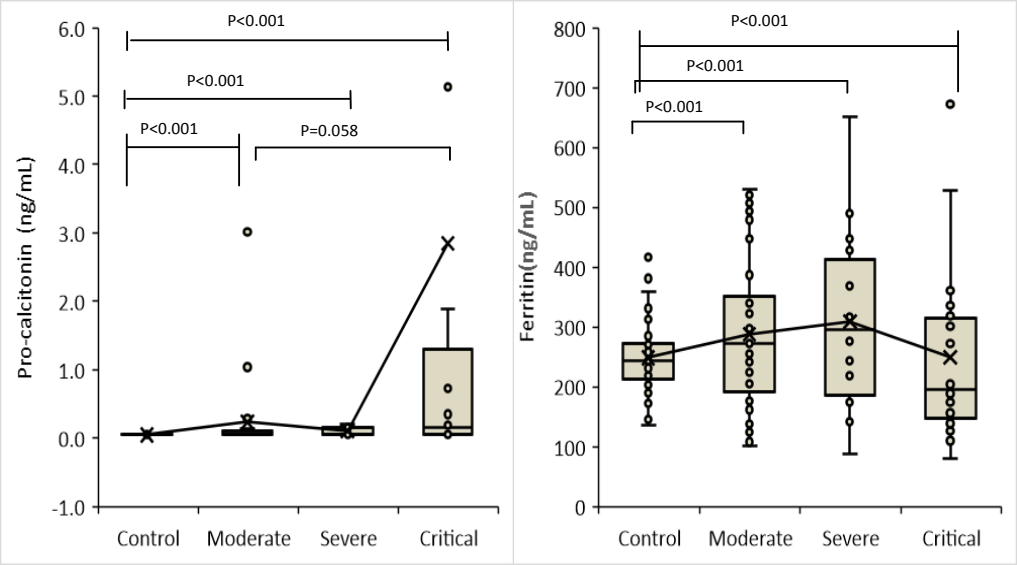

Figure 1. Laboratory findings of COVID-19 patients based on disease's severity: Hospitalized COVID-19 patients were divided into three groups based on the disease severity: the moderate (n=43), severe (n=15), and critical (n=21). The levels of laboratory parameters, including white blood cells count, inflammatory markers, and cytokines were compared between groups and a healthy control group (n=68). A) White blood cell subsets frequency is presented as a concentration of thousands of cells per microliter of blood. The bar chart is drawn using the mean of data. Error bars denote the standard deviation. B) Quantification of inflammatory and anti-inflammatory cytokines and inflammatory and infection mediators, including c-reactive protein, pro-calcitonin, and ferritin are presented. For the boxplots, the center is drawn based on the median of the measurement, while the lower and upper bounds of the box correspond to the first and third percentile. Whiskers beyond these points represented 1.5 × the interquartile range. Between assessed parameters, the result of items that had a significant difference is presented in this figure. *p*-value was measured using t-test and Mann-Whitney test for normally distributed and non-normally distributed data, respectively. $p<0.05$ was regarded as statistically significant.

Increased serum IL-6, IL-1Ra, and IL-8 were significantly prevalent in COVID-19 patients compared to the HC group ($p<0.001$, $p=0.006$, and $p=0.013$, respectively). This increase in serum IL-6 and IL-1Ra was especially significant in critical patients compared to the moderate group ($p=0.008$ and $p=0.010$, respectively) (Table 2). Of note, the Median of serum IL-6 and IL-8 were significantly elevated in COVID-19 subjects, especially in those classified as critical (Figure 1). Besides, increased serum IL-10 levels were also reported to be significantly more prevalent in severe and critical patients compared to moderate patients ($p=0.025$ and $p=0.017$, respectively).

Immunology Related Biomarkers are the Strongest Mortality Risk Factors

To associate biomarkers with mortality risk, we performed the univariate analysis for all the assessed factors between survived and non-survived patients with COVID-19 (Table 3). Increased ferritin, PCT, troponin I, D-dimer, WBC, neutrophil, NLR, IL-1 β, IL-6, IL-10, and IL-1Ra were associated with the increased mortality of COVID-19 patients ($p= 0.011$, $p=0.002$, $p=0.004$, $p=0.010$, $p=0.008$, $p=0.001$, $p=0.040$, $p=0.036$, $p<0.001$, $p=0.006$, $p=0.004$, $p=0.027$ and $p=0.008$, respectively) (Table 3). Decreased troponin-I was also reported to be associated with the mortality risk of COVID-19 patients ($p<0.001$) (Table 3).

To assay the prognostic value and the appropriate cut-off points of the variables and also to find a statistically significant association with the mortality risk of patients, we drew the receiver operating characteristic (ROC) curves. Variables with a specificity and sensitivity of more than 60% are displayed in Figure 2. Troponin-I with 16.95 ng/mL cut-off point had the highest specificity (95%) and sensitivity (80%) among the assessed variables

Table 3. The laboratory findings of survived and dead patients with COVID-19

	Cut-off	Survivor	Non-survivor	*p*	OR	95%CI
Blood sugar (mg/dL)	≥115	19/41(46%)	3/8(38%)	0.646	0.695	0.146 - 3.298
Urea (mg/dL)	≥45	26/50(52%)	7/14(50%)	0.895	0.923	0.282 - 3.021
Creatinine (mg/dL)	≥1.4	9/52(17%)	2/12(17%)	0.958	0.956	0.178 - 5.125
Uric Acid (mg/dL)	≥7.2	5/38(13%)	1/9(11%)	0.869	0.825	0.084 - 8.08
Triglycerides (mg/dL)	≥200	12/38(32%)	1/8(13%)	0.276	0.310	0.034 - 2.805
Phosphorus (mg/dL)	≥4.5	5/44(11%)	0/9(0%)	0.288	--	
	≤2.6	10/44(23%)	3/9(33%)	0.500	1.700	0.359 - 8.049
Total Bilirubin (mg/dL)	≥1.2	20/40(50%)	4/9(44%)	0.763	0.800	0.187 - 3.423
SGOT (U/L)	≥37	24/57(42%)	8/12(67%)	0.121	2.750	0.742 - 10.196
SGPT (U/L)	≥41	25/56(45%)	4/12(33%)	0.472	0.620	0.167 - 2.3
LDH (U/L)	≥480	49/58(84.5%)	13/14(92.9%)	0.416	2.388	0.277 - 20.592
ALP (U/L)	≥306	5/56(9%)	1/12(8%)	0.947	0.927	0.098 - 8.743
CPK (U/L)	≥190	7/50(14%)	2/12(17%)	0.814	1.229	0.221 - 6.83
CRP (U/L)	≥6	53/54(98%)	13/14(93%)	0.296	1.327	0.142 - 12.366
	≥10	49/54(91%)	13/14(93%)	0.804	1.667	0.427 - 6.511
Ferritin (ng/mL)	≥365	48/64(75%)	15/18(83%)	0.459	4.833	1.329 - 17.577
	≥1500	6/64(9%)	6/18(33%)	**0.011***	8.200	1.897 - 35.44
Procalcitonin (ng/mL)	≥0.5	5/46(11%)	6/12(50%)	**0.002***	12.400	1.777 - 86.504
Troponin I (ng/mL)	≥38	2/40(5%)	5/10(50%)	**0.004***	19.000	2.881 - 125.313
	≤15	38/40(95%)	2/10(20%)	**<0.001***	0.013	0.002 - 0.108
D-dimer (µg/L)	≥1000	27/60(45%)	13/16(81%)	**0.010***	5.296	1.367 - 20.522
WBC (10^3/µL)	≥11	12/63(19%)	9/18(50%)	**0.008***	4.250	1.39 - 12.995
	≤4.1	10/63(16%)	0/18(0%)	0.071	--	
RBC (10^6/µL)	≥5.3	10/63(16%)	2/18(11%)	0.616	0.663	0.131 - 3.34
	≤4.3	28/63(44%)	12/18(67%)	0.096	2.500	0.833 - 7.501
Hb (10^3/µL)	≤13.5	43/63(68%)	14/18(78%)	0.435	1.628	0.475 - 5.577
HCT (10^3/µL)	≥45	7/63(11%)	1/18(6%)	0.486	0.471	0.054 - 4.099
	≤38	35/63(56%)	9/18(50%)	0.676	0.800	0.28 - 2.284

Platelets (10^3/μL)	≥450	7/63(11%)	2/18(11%)	>0.999	1.000	0.189 - 5.295
	≤150	10/63(16%)	4/18(22%)	0.530	1.514	0.412 - 5.559
Neutrophil (10^3/μL)	> 6.3	41/63(65.1%)	7/18(38.9%)	**0.046***	8.125	2.128 - 31.024
Lymphocyte (10^3/μL)	≤1	39/63(61.9%)	12/18(66.7%)	0.712	5.263	1.112 - 24.902
NLR (%)	≥3.53	40/63(63.5%)	16/18(88.9%)	**0.040***		
Monocyte (10^3/μL)	> 0.5	29/63(46%)	11/18(61.1%)	0.259	0.123	0.036 - 0.424
Eosinophil (10^3/μL)	≤1	38/63(60.3%)	8/18(44.4%)	0.231	8.500	1.058 - 68.29
TNF α (ng/L)	≥8.1	10/52(19%)	5/12(42%)	**0.098**	3.000	0.786 - 11.445
IL-1 β (ng/L)	≥5	0/52(0%)	1/12(8%)	**0.036***	--	--
IL-6 (ng/L)	>6	57(79.2%)	27(96.4%)	**0.035***	0.045	0.019-0.499
IL-10 (ng/L)	≥9.1	5/52(10%)	5/12(42%)	**0.006***	6.714	1.541 - 29.264
IL-1Ra (ng/L)	≥1	9/51(17.6%)	7/12(58.3%)	**0.004***	6.533	1.686 - 25.322
IL-8 (ng/L)	≥62	7/44(16%)	4/13(31%)	0.233	2.349	0.563 - 9.799

Data are presented as n/N (%), where N is the total number of patients with available data. Statistical analysis was performed using the χ^2 test. *p*, comparison between healthy control and COVID-19 patients; * $p<0.05$ was regarded as statistically significant.
COVID-19, coronavirus disease 2019; LDH, lactate dehydrogenase; SGOT, serum glutamic oxaloacetic transaminase or aspartate aminotransferase (AST); SGPT, serum glutamic pyruvic transaminase or alanine aminotransferase (ALT); ALP, alkaline phosphatase; CPK creatine phosphokinase; CRP, C-reactive protein, WBC, white blood cell; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; NLR, neutrophil-lymphocyte ratio; IL, interleukin; TNF-α, tumor necrosis factor-alpha; IL-1Ra, interleukin-1 receptor antagonist; mg/dL, milligrams per deciliter; ng/mL, nanograms per milliliter; μg/L, micrograms per liter; U/L, unit per liter.

(AUC=0.900, 95%CI: 0.798-0.999, $p<0.001$). IL-6 with 60.6 ng/L (ACU=0.792, 95% CI: 0.663-0.921, $p<0.001$) were also shown to be with the specificity for 92.2% and the sensitivity for 61.1%. NLR specificity and sensitivity were 83.3% and 66.7% for mortality prediction, respectively (ACU=0.787, 95%CI: 0.654-0.920, $p<0.001$). Additionally, PCT with estimated 0.145 ng/mL cut-off point was shown to have the specificity of 71.7% and sensitivity of 75% (ACU=0.777, 95% CI: 0.615-0.940, $p=0.003$) for the death outcome. Finally, D-dimer specificity and sensitivity were reported 75% and 68.8% for death prediction, respectively (ACU=0.744, 95% CI: 0.612-0.876, $p=0.003$) (Figure 2).

Elevation of NLR and Serum Inflammatory Biomarkers and its Strong Correlation with Elevated IL-6 and IL-1β

IL-6 showed strong correlation with IL-1β, IL-10, IL-1Ra, IL8 ($p= 0.016$, $p=0.001$, $p<0.001$ and $p=0.004$) and also with CRP, PCT and troponin-I ($p=0.006$, $p<0.001$ and $p=0.006$) but not TNF-α ($p=0.713$) (Table 4). Besides, IL-6 showed a significant positive correlation with NLR ($p=0.025$) (Table 4).

NLR is also shown to have a significant positive correlation with ferritin, PCT, troponin-I, D-dimer, IL-β, and IL-10 ($p=0.002$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.009$ and $p=0.008$, respectively) (Tables 4 and 5).

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Table 4. Correlation of cytokines with immunological factors

	TNF-α		IL-1 β		IL-6		IL-10		IL-1Ra	
	P	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***
TNF-α	-	-	**<0.001**	0.440**	0.713	0.047	**0.001**	0.416**	0.082	0.221
IL-1 β	**<0.001**	0.440**	-	-	**0.016**	0.300*	**<0.001**	0.476**	**0.001**	0.407**
IL-10	**0.001**	0.416**	**<0.001**	0.476**	**0.001**	0.390**	-	-	**0.050**	0.248
IL-1Ra	0.082	0.221	**0.001**	0.407**	**<0.001**	0.594**	**0.050**	0.248	-	-
IL-8	0.503	0.096	**<0.001**	0.489**	**0.004**	0.349**	0.098	0.234	**0.004**	0.404**
CRP	0.452	-0.102	0.318	-0.135	**0.006**	0.317**	0.975	0.004	0.863	0.024
Ferritin	0.215	0.157	**0.039**	0.259*	0.124	0.161	0.114	0.199	0.099	0.210
Procalcitonin	0.586	0.078	0.148	0.205	**<0.001**	0.528**	**0.002**	0.427**	**0.007**	0.374**
Troponin I	0.327	0.153	0.085	0.266	**0.006**	0.362**	**0.027**	0.336*	**0.002**	0.474**
D-dimer	0.198	0.164	**0.012**	0.313*	0.253	0.132	**<0.001**	0.431**	**0.021**	0.292*
WBC	0.101	0.207	**0.026**	0.278*	0.956	0.006	**0.038**	0.260*	**0.014**	0.309*
Neutrophil	0.260	0.122	0.173	0.147	0.210	0.109	0.084	0.186	**0.028**	0.237*
Lymphocyte	0.182	-0.144	0.093	-0.181	0.062	-0.162	**0.013**	-0.266*	0.900	-0.014
NLR	0.104	0.176	**0.009**	0.278*	**0.025**	0.194*	**0.008**	0.283*	0.095	0.181

r: Spearman correlation; *, $p<0.05$ was regarded as statistically significant; **, $p<0.001$.
Abbreviations: *p*, *p*-value; CRP, C-reactive protein; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; IL, interleukin; TNF-α, tumor necrosis factor-alpha; IL-1Ra, interleukin-1 receptor antagonist.

Table 5. Correlation of inflammatory markers with immunological factors

	CRP		Ferritin		Procalcitonin		Troponin I		D-dimer	
	P	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***
Ferritin	0.362	0.108	-	-	**0.003**	0.386*	0.094	0.224	**0.007**	0.307*
Procalcitonin	**0.026**	0.312*	**0.003**	0.386*	-	-	**<0.001**	0.699**	**0.017**	0.322*
Troponin I	**0.047**	0.291*	0.094	0.224	**<0.001**	0.699**	-	-	**0.009**	0.365**
D-dimer	0.812	-0.03	**0.007**	0.307*	**0.017**	0.322*	**0.009**	0.365**	-	-
WBC	0.440	-0.092	0.164	0.150	0.108	0.213	0.062	0.253	**<0.001**	0.501**
Neutrophil	0.958	-0.005	**0.016**	0.211*	0.059	0.211	**0.002**	0.334*	**<0.001**	0.522**
Lymphocyte	0.194	-0.125	**0.039**	-0.181*	**0.009**	-0.289*	**0.029**	-0.244*	0.178	-0.131
NLR	0.296	0.101	**0.002**	0.263*	**<0.001**	0.359*	**<0.001**	0.462*	**<0.001**	0.478**

r: Spearman correlation; *, $p<0.05$ was regarded as statistically significant; **, $p<0.001$.
Abbreviations: *p*, *p*-value; CRP, C-reactive protein; WBC, white blood cell; NLR, neutrophil to lymphocyte ratio

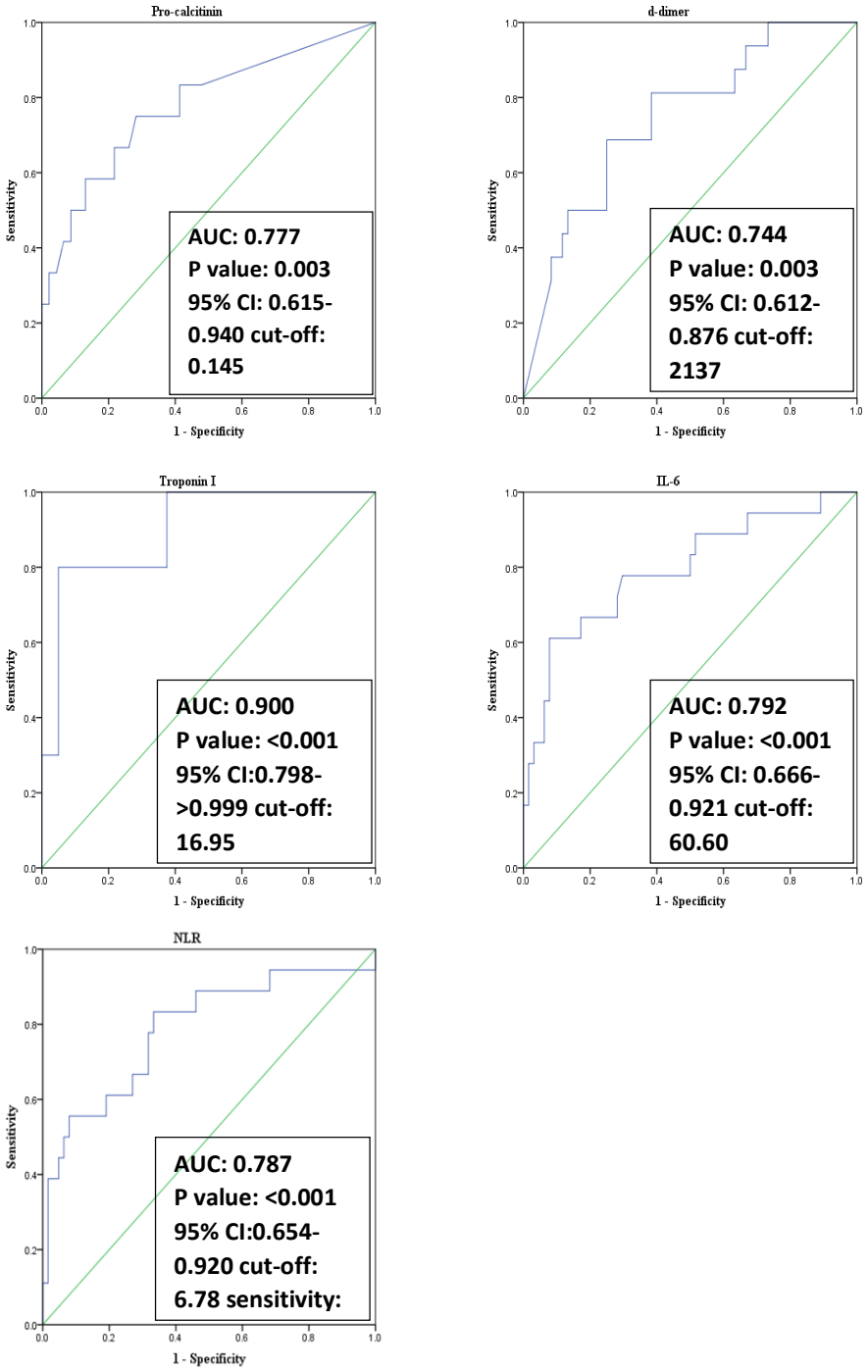

Figure 2. The receiver operating characteristic (ROC) curve of procalcitonin, interleukin-6 (IL-6), D-dimer, troponin I and, neutrophil to lymphocyte ratio (NLR) for the prediction of fatal outcome in hospitalized patients with COVID-2019
Abbreviations: AUC, the area under the curve; CI, confidence interval.

DISCUSSION

A comprehensive understanding of the COVID-19 immunopathogenesis contributes to its better management. In this regard, finding prognostic and predictive biomarkers can help us to determine the severity of the disease correctly. In this study, different laboratory factors of COVID-19 hospitalized patients (in Tehran City, Iran) were investigated based on the disease severity and mortality. The majority (78.9%) of the patients were male, with the mean (SD) age of 59.14 (14.81) years. Additionally, deceased cases were significantly prevalent among critical as compared to moderate and severe cases of COVID-19 (both $p<0.001$). Males with older age (>55 years) with at least one or two comorbidities ($p<0.001$ for both) tended to develop more severe cases presenting with chest pain ($p=0.032$) and low SpO_2 ($p=0.030$), mostly resulting in the death of the critical patients. Consistently, the highest fatality was observed in men classified as elderly (>50 years) with underlying diseases.[12-14] The presence of the above-mentioned underlying diseases is considered an essential factor in disrupting the results of serum biochemical measures. Diabetes, often accompanied by hyperuricemia, is one of the essential underlying disorders in these patients. This condition increases the risk and severity of COVID-19 in individuals. Hyperglycemia and hyperuricemia in patients with COVID-19, which was also observed in the present study, as a promoter of hyper inflammation through increasing oxidative stress and altering the inflammatory and anti-inflammatory cytokines balance, increases the severity of COVID-19 and increases the mortality in infected patients.[15-17] It is noteworthy to mention that uric acid activates the NLRP3 inflammasome increasing IL-1β, IL-18, IL-6, and TNF-α, thus explaining the importance of risk factors for COVID-19 severity, including diabetes and kidney disease.[18] Renal impairment in chronic and acute forms of kidney diseases affects the severity and survival rate of patients with COVID-19. Similar to the present study's findings, an increase in blood urea nitrogen (BUN) and creatinine in COVID-19 was reported in previous studies.[19,20] Even a significant increase was reported in the deceased group than the recovered patients, which the latter was not reported in the present study. It should be noted that during COVID-19 infection, a circulating virus could damage the renal resident cells resulting in elevated levels of BUN, serum creatinine, uric acid, etc.[21,22]

Unlike previous factors, a decrease in blood phosphorus in patients with COVID-19 is a valuable finding since it contributes to adenosine triphosphate (ATP) synthesis and the metabolism of energy. In a previous study, Xue et al showed that serum phosphorus levels were positively correlated with the absolute value of lymphocytes, and phosphorus supplementation improved the immune level and promoted the recovery of COVID-19 patients who classified as severe with lowered serum phosphorus.[23].

In addition to all the above-mentioned serum biochemical factors, significantly elevated SGOT, SGPT, and total bilirubin levels were reported in the COVID-19 patients of the current study. It has been proven that impaired liver function with elevated liver cell injury markers (SGOT and SGPT) in the serum of patients with COVID-19 is a common finding of these patients even on admission.[24] Of note, Wang et al. reported the correlation between the severity of COVID-19 and total bilirubin, suggestive of the predictive value of this marker for the patients' condition.[25] These findings emphasize the necessity of considering the monitoring and treatment of the liver in COVID-19. Moreover, significantly elevated LDH in COVID-19 patients, especially in critical patients, is similar to the findings of elevated LDH in SARS and MERS.[26,27] In this regard, Wu et al. also validated the efficiency of LDH evaluation in COVID-19 caused pneumonia for early intervention.[28] Finally, troponin-I as a cardiac tissue-specific marker was shown to be significantly elevated in non-survivor critical COVID-19 subjects, which classifies people with underlying cardiovascular diseases among those with increased risk for death.

The above-mentioned markers are more helpful in following up the patient's condition based on the organs involved. At the same time, CRP and serum ferritin as factors of the inflammatory status can be used as early markers in predicting the severity of COVID-19.[29,30] This clue results from the nature of CRP, which is produced mainly by the liver following infection to play a crucial role in complement activation and also activating apoptosis, phagocytosis, nitric oxide release, and cytokine production, especially IL-6, IL-8, MCP-1, and TNF-α.[31] While the primary role of ferritin is to inhibit iron from the production of tissue-damaging radicals and to maintain it for the synthesis of

hemoglobin and other vital processes, apart from iron content, ferritin itself can potentiate cytokine cascades of nuclear factor kappa-B (NF-κB).[32,33] The association of elevated serum CRP with the COVID-19 severity and the need for ICU care but not the survival of patients has already been reported (34). This is while serum ferritin levels of COVID-19 patients were associated with the disease severity, mortality, and even the development of ARDS.[34] CRP and ferritin cannot be used independently to predict the outcome of patients since these factors are being affected by age, gender, BMI, smoking, blood pressure, and so on.[35,36] In addition to serum CRP and ferritin, there is increasing evidence of elevated serum PCT and D-dimer with the poor outcome of the COVID-19 patients.[34] These findings are consistent with the reported data, representing significantly elevated PCT and D-Dimer in critical patients. Besides, the elevation of ferritin, PCT, and D-dimer was also significantly more prevalent among non-survivors, suggesting the poor outcome of patients with elevation of these factors. Moreover, a significant and robust correlation between CRP, ferritin, PCT, D-dimer, and troponin-I and a strong and significant correlation of these factors with inflammatory and anti-inflammatory immunological factors measured in the present study, especially IL-6, was reported. These findings can be well explained in the context of the cytokine storm in advanced stages of the disease with hyper inflammation resulting in multi-organ failure.[37]

The cytokine storm following cytokine release syndrome in COVID-19, which has been identified as a significant cause of ARDS, mainly depends on IL-6.[38,39] In viral infections, the production of proinflammatory cytokines such as IL-1 and TNF-α occurs after detection of viral RNA as a pathogen-associated molecular pattern (PAMPs) by toll-like receptors (TLR).[40] These cytokines stimulate the production of IL-6 by stimulating various cells, including fibroblasts, mesenchymal cells, etc. After the occurrence of tissue damage, the release of the danger-associated molecular patterns (DAMPs) and the activation of the coagulation cascade further increase IL-6 production.[41,42] Increased serum IL-6 with the severity of the disease was accompanied by its positive correlation with serum levels of IL-1 β, IL-8, IL-10, and IL-1Ra. In other words, measured cytokine profile during the active phase of the disease indicates the presence of both inflammatory and anti-inflammatory responses to SARS-CoV-2 infection. These findings agree with the fact that IL-6 increases the synthesis of IL-8,[43] IL-1Ra, and IL-10.[44]

The imbalance of cytokines and chemokines in COVID-19 affects many factors, especially white blood cells.[45] Increased WBC, along with elevated NLR, was prevalent among critical COVID-19, in which elevated NLR was indicative of poor outcome. Elevated NLR resulted from the increased neutrophil count, with the disease severity resulted from significantly increased neutrophil. Also, decreased lymphocyte count was significantly correlated with inflammation manifested by elevated ferritin, PCT, D-dimer, IL-1β, and IL-6. All these data add value to the claimed specificity and sensitivity of increased NLR for predicting the severity and outcome of COVID-19.[46]

Additionally, increased circulatory monocytes in critical subjects - may be explained by recruiting monocytes from the lungs, thus promoting the ARDS.[47] Moreover, significantly prevalent eosinopenia in COVID-19 subjects is consistent with the findings of previous studies[48] in which this characteristic was regarded to be unique for COVID-19 compared to other types of pneumonia. Finally, decreased RBC, Hb, HCT, and platelet values in COVID-19 subjects compared to HC have been previously shown to be more significant in severe subjects.[49] This finding suggests the impaired erythropoiesis[50] as a consequence of immune damage triggered bone marrow suppression. This condition has been accompanied by elevated morphological parameters of RBCs as an essential indicator of compensatory erythroid hyperplasia.[49]

Lack of available clinical data for fourteen patients limited us to put them in a proper group based on the disease severity and outcome. Herein, the sample size was low in comparison of cytokines and it maybe affects the significance of results.

The current study comprehensively examined the clinical condition of COVID-19 patients based on the severity of the disease and their outcome with routine and available tests in medical laboratories. Firstly, it is essential to pay attention to the age and sex of the patient, the underlying diseases, and blood oxygen pressure. Secondly, a simple CBC test with careful attention to RBC parameters, WBC count, and NLR, along with eosinophil count, helps predict the patient's condition. Finally, elevated serum CRP, LDH, PCT, D-dimer, and IL-6 should draw attention to the need for ICU.

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CONFLICT OF INTEREST

All authors declared no conflict of interest.

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