Research in Molecular Medicine

The Association Between Laboratory Biomarkers and Clinical Features in COVID-19 Pneumonia: A Potential Tool for Predicting Disease Prognosis and Severity

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ABSTRACT

Background: The COVID-19 pandemic has rapidly spread and remained poorly understood by clinicians. The present work aimed to study the association between laboratory biomarkers, prognosis, and disease severity.

Materials and Methods: This is a single-center cohort study. We included young patients admitted at Razi Hospital, Ghaemshar City, Iran, from April 2020 to June 2020, whose diseases were confirmed with reverse transcription real time-PCR (rRT-PCR) test. Laboratory biomarkers were analyzed on the same day of inpatient service and after five days of hospitalization. The patients' results and the outcomes were compared with those of the control group.

Results: In the present study, 70 patients were investigated; 53 were discharged, and 17 died. A significant correlation was observed between patients and healthy subjects in some laboratory biomarkers: C-reactive protein (CRP), lactate dehydrogenase (LDH), total protein level, albumin level, and absolute lymphocyte count. Furthermore, CRP, LDH, total protein, albumin, absolute lymphocyte count, 25-OH vitamin D, interleukin (IL)-6, ferritin, and D-dimer levels in patients with different outcomes had significant correlations. High CRP, LDH, IL-6, ferritin, and D-dimer were predictive of mortality (area under the curve >0.70), as were low absolute lymphocyte count and 25-OH vitamin D. After adjusting age, CRP, albumin, WBC, D-dimer, LDH, and 25 OH-vitamin D, the final model of multiple binary logistic regressions with IL-6 and ferritin had high accuracy for the prediction of fatal outcome.

Conclusion: This finding would facilitate the early stratification of hospitalized patients with COVID-19 and help make clinical decisions.

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Research in Molecular Medicine

Introduction

A viral pneumonia emerged in Wuhan, China, in December 2019, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The virus affects all organs of the body, especially the lungs [2]. By February 2020, the World Health Organization (WHO) changed the disease name to novel coronavirus disease 2019 (COVID-19) [3]. Fast spread and person-to-person transmission has made SARS-CoV-2 a global health threat with the pandemic's socioeconomic effects [4, 5]. The clinical manifestations of the disease typically begin in symptomatic patients in less than a week, consisting of fever (high temperature >37.3°C), cough, myalgia, nasal congestion, diarrhea, and fatigue. Pneumonia often occurs during a symptomatic infection in the second or third week, and in some patients, the disease is associated with a hyper-immune reaction and cytokine storm [6, 7]. The severity of COVID-19 infection appears to be diverse, from none to acute respiratory distress syndrome (ARDS), multi-organ failure, and finally, death [8]. The overall rate of mortality because of COVID-19 is around 1% to 3% [9]. In comparison, the various mortality rates reported in hospitalized patients range from 6% to 34% [10-12], and it can reach 50% in intensive care units [13].

Recent studies have shown that some biochemical and hematological parameters in COVID-19 patients are distinctly altered [14, 15], and certain laboratory biomarkers are associated with poor prognosis [16]. A few studies have reported the clinical manifestations of hospitalized patients with SARS-CoV-2 and proposed risk factors regarding its mortality [17].

The present study aimed to compare laboratory biomarkers between patients and healthy subjects and analyzed the relationship between these markers and the discharge or death of COVID-19 patients. The results of this study and similar ones could provide a standard protocol for laboratory tests to predict the prognosis and mortality of the patients.

Materials and Methods

Patients and controls

Seventy patients (40 men and 30 women, 23-36 years old) affected with COVID-19 (confirmed with reverse transcription real time-PCR [rRT-PCR] test) who were admitted to the Razi Hospital (Ghaemshahr City, Iran) affiliated to Mazandaran University of Medical Sciences from April 2020 to June 2020 were recruited. Also, 48 healthy individuals (35 men and 13 women, between 20-40 years old) without COVID-19 symptoms during the last 2 months and rRT-PCR negative for SARS-CoV-2 were selected as controls. The included patients were categorized into two groups based on their clinical outcomes: The first group with an acceptable prognosis of the disease (discharge after recovery) and the second group with a poor prognosis (death). The exclusion criteria were a history of underlying disease like diabetes mellitus, chronic kidney disease (CKD), cardiovascular disease (CVD), malignancy, or a history of pulmonary involvement such as tuberculosis or chronic obstructive pulmonary disease (COPD). All individuals signed an informed consent form.

Laboratory measurements

Routine medical laboratory parameters such as C-reactive protein (CRP) level, total protein level, albumin level, lactate dehydrogenase (LDH) activity, total IgG level, total IgM level, 25-OH vitamin D level, magnesium (Mg) level, white blood cells (WBC) count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC) and platelets count were determined. All laboratory tests were performed on the hospitalization day. After five days of hospitalization, the plasma levels of interleukin-6 (IL-6) as a cytokine storm and inflammation index, D-dimer as a coagulopathy marker, and ferritin indicating acute phase status were analyzed. All patients were followed until their discharge or death.

CRP, LDH, total protein, albumin, Mg, total IgM, and total IgG were measured by a Hitachi 912 device (Hitachi, Japan) using either a spectrophotometric assay (LDH), a colorimetric assay (total protein and albumin) or an immunoturbidimetric assay (CRP, total IgM, and total IgG). For the detection of 25 OH-vitamin D level, the HPLC (High-performance liquid chromatography) method (Agilent, USA) was applied. WBC, ANC, ALC, and platelets count were analyzed using Sysmex XT 1800i (Sysmex, Japan). COBAS INTEGRA 400 plus (Roche Diagnostic, Basel, Switzerland) was used to measure IL-6, D-dimer, and ferritin levels. The rRT-PCR was performed on a LightCycler 96 (Roche Diagnostic, Basel, Switzerland) using the Pishtaz Teb provided 2019-nCoV real-time reverse transcriptional PCR Kit. RNA purification was performed using the MagCore Compact as an automated nucleic acid extractor (MagCore, Singapore).

Statistical analysis

The association between disease outcomes and blood biomarkers was analyzed using the chi-square and Mann-Whitney U tests. Multivariate logistic regression was performed to assess the ability of demographic and blood marker features to predict the disease outcomes. Receiver operating characteristic curve (ROC) analysis was applied to evaluate the prediction model. The SPSS software, version 22 (IBM Corp) was used for the research data analysis. The data normality was assessed by the Kolmogorov–Smirnov test. A two-sided P<0.05 was considered statistically significant for all tests.

Results

The demographic information and major clinical characteristics of patients are summarized in Table 1. The median age was 29 (IQR, 26-33 y), and 40 patients (57.1%) were men. The most common symptoms on admission day were fatigue (90.0%), fever (81.4%), dry cough (70.0%), dyspnea (60.0%), myalgia (47.1%), diarrhea (33.8%), abdominal pain (24.2%), headache (14.2%), and vomiting (12.8%); 53 out of the 70 investigated patients were discharged, and 17 patients died.

The rise in laboratory biomarkers in patients with COVID-19

The comparison of laboratory biomarkers levels between patients with COVID-19 and healthy controls showed that CRP, LDH, total protein, albumin, WBC, and absolute lymphocyte count were significantly different between the two groups (P<0.05), (Figure 1). Total IgM was significantly higher in patients than healthy individuals (P=0.043, weak correlation). In contrast, total IgG, platelets count, and absolute neutrophil count did not show a statistically significant difference (P>0.05), (Table 2).

Comparison of laboratory biomarkers and clinical outcome

As shown in Figure 1, the laboratory biomarkers such as CRP, LDH, total protein, albumin, WBC, and absolute lymphocyte count were significantly different among patients with complete recovery and death outcomes (P<0.05). Indeed, patients who eventually died had higher levels of laboratory biomarkers. No significant correlations were observed between total IgG, platelets count, and absolute neutrophil count in patients with different outcomes (P>0.05), (Table 3). It is considerable that the serum level of 25-OH vitamin D was significantly lower in patients who died (P=0.003), (Figure 2a), while serum Mg level was not different in two fates (P=0.71), (Table 3).

Table 1. Demographics and characteristics of patients (infected with COVID-19) and healthy controls

Characteristics		No. (%)			
		Patients (n=70)	Discharge (n=53)	Death (n=17)	Healthy Controls (n=48)
Age (y)	Median	29	31	28	30
Sex	Male	40(57.1)	30(56.6)	10(58.8)	35(72.9)
	Female	30(42.9)	23(43.4)	7(41.2)	13(27.1)
Signs and symptoms	Fever	57(81.4)	44(83.1)	13(76.5)	-
	Fatigue	63(90.0)	52(98.1)	11(64.7)	-
	Myalgia	33(47.1)	27(50.9)	6(35.2)	-
	Dry cough	49(70.0)	37(69.8)	12(70.5)	-
	Dyspnea	42(60.0)	28(40.0)	14(82.3)	-
	Diarrhea	23(33.8)	18(33.9)	5(29.4)	-
	Headache	10(14.2)	6(11.3)	4(23.5)	-
	Vomiting	9(12.8)	7(13.2)	2(11.7)	-
	Abdominal pain	17(24.2)	12(22.6)	5(29.4)	-

Table 2. Variable measured in patients (infected with COVID-19) and healthy controls

Characteristics	Patients (n=70)		Healthy Controls (n=48)		P
Variables	Median	IQR	Median	IQR	
Age (y)	29	26-33	30	28-33	=0.388
CRP level (mg/dL)	55.5	23.0-91.0	9.5	6-11.7	<0.0001
Total protein level (g/dL)	6.4	6.1-6.8	7.2	7.0-7.6	<0.0001
Albumin level (g/dL)	5.0	4.9-5.4	5.8	5.5-6.1	<0.0001
LDH level (U/L)	712	467-951	296	225-331	<0.0001
Total IgM level (U/L)	100	53-148	122	63-157	=0.043
Total IgG level (U/L)	1229	1036-1385	1269	1138-1365	=0.146
WBC count (/µL)	5500	4100-6700	6630	5400-7400	=0.002
Neutrophil count (/µL)	3240	2400-4100	3662	2800-4375	=0.079
Lymphocyte count (/µL)	2024	1000-2800	2722	2300-3100	<0.0001
Platelet count (*1000 cells/µL)	276	184-366	242	178-316	=0.074

Table 3. Variables measured in patients discharged and died

Characteristics	Patients (n=70)				P
	Discharge (n=53)		Death (n=17)		
Variable	Median	IQR	Median	IQR	
Age (y)	31	26-33	28	26-32	=0.235
CRP level (mg/dL)	49	11-90	76	58-98	=0.013
Total protein level (g/dL)	6.5	6.2-6.9	6.2	6.0-6.6	=0.028
Albumin level (g/dL)	5.1	4.9-5.4	4.8	4.4-5.2	=0.032
LDH level (U/L)	625	354-868	981	732-1150	<0.0001
Total IgM level (U/L)	102	53-153	92	51-120	=0.81
Total IgG level (U/L)	1246	1018-1392	1178	1038-1281	=0.35
WBC count (/µL)	5800	4400-7100	4400	3400-5200	=0.005
Neutrophil count (/µL)	3318	2350-4100	2994	2400-3650	=0.38
Lymphocyte count (/µL)	2264	1650-3000	1276	650-2000	=0.001
Platelet count (*1000 cells/µL)	276	177-367	276	199-333	=0.84
25-OH vitamin D level (ng/mL)	20.3	13.1-35.0	10.7	7.4-20.1	=0.003
Mg level (mEq/L)	1.7	1.5-2.1	1.8	1.6-1.9	0.71
IL-6 level (pg/mL)	83	14-117	529	322-744	<0.0001
D-Dimer level (ng/mL)	643	332-4113	6225	2502-8533	<0.0001
Ferritin level (ng/mL)	184	43-662	749	432-1290	<0.0001

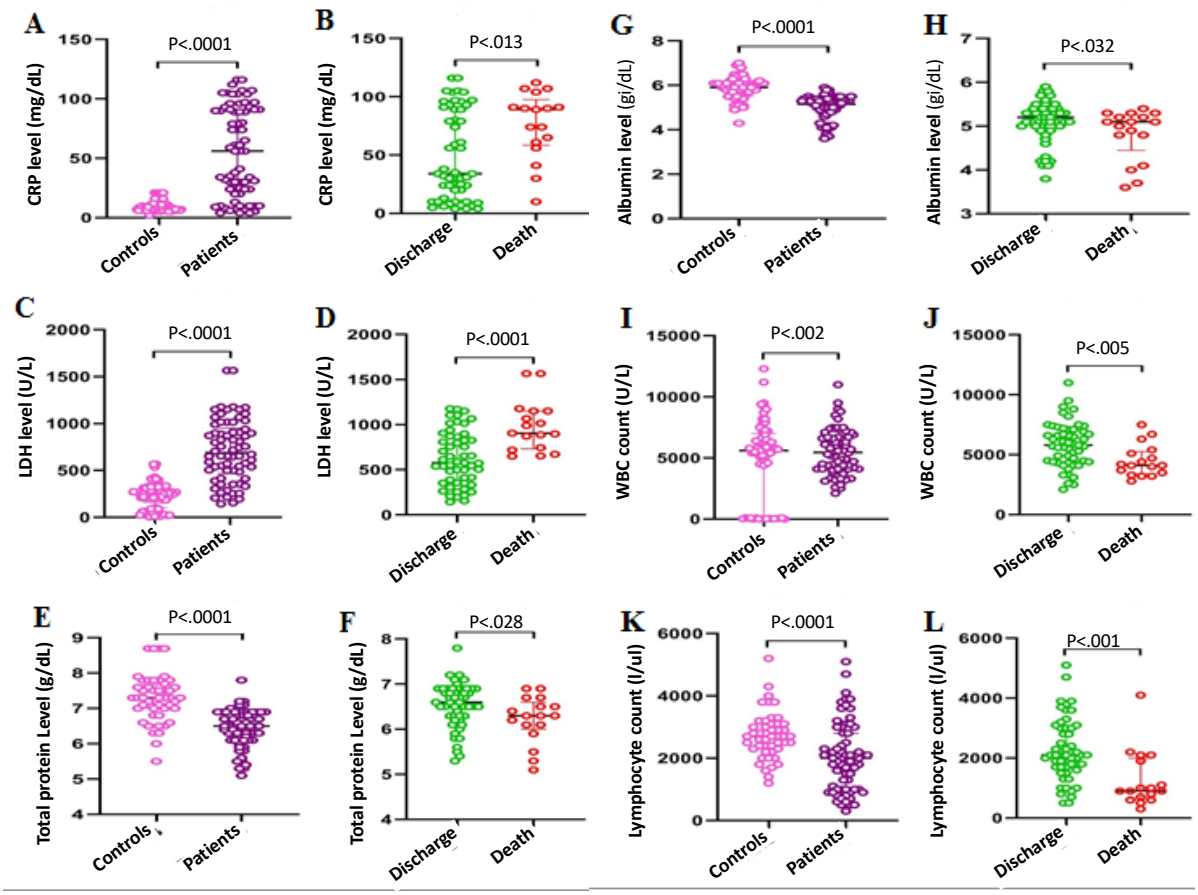

Figure 1. 25-OH vitamin D and magnesium (Mg) levels in patients with different outcomes

A) Comparison of 25-OH vitamin D levels among different subgroups, B) Comparison of Mg level among different subgroups

Note: The Mann-Whitney U test was used to compare the two groups.

Comparing inflammation markers and clinical outcomes

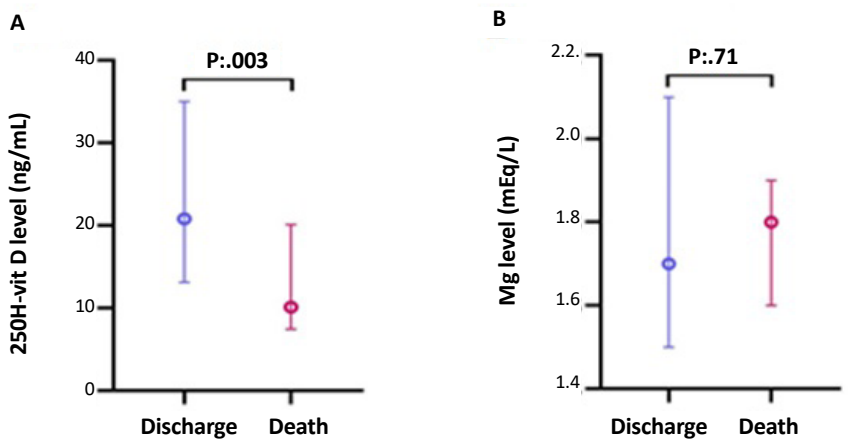

Figure 2. Scatter plot analysis of laboratory biomarkers in patients (with different outcomes) and healthy controls

A-L) Comparison of CRP level, LDH level, total protein level, albumin level, WBC count and absolute lymphocyte count between the controls and patients and among different subgroups

Note: The Mann-Whitney U test was used to compare the two groups.

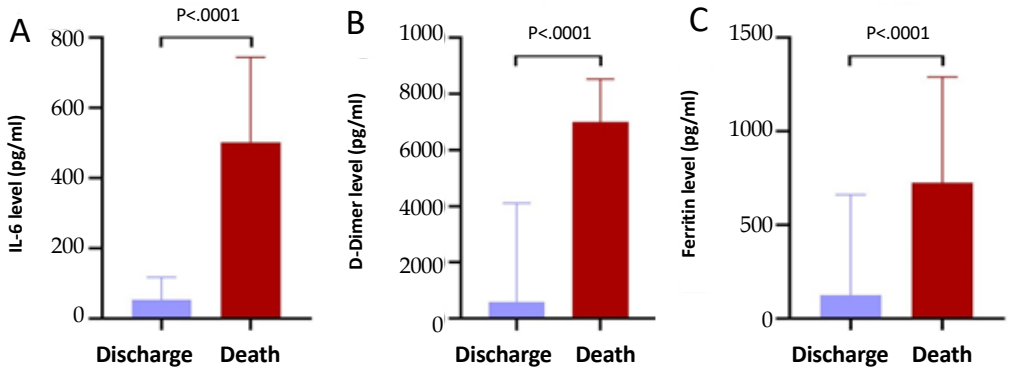

Figure 3. Interleukin (IL)-6, D-Dimer, and ferritin levels in patients with different outcomes
A-C) Comparison of IL-6, D-Dimer, and Ferritin levels among different subgroups.
Note:The Mann-Whitney U test was used to compare the two groups.

The results showed higher levels of IL-6, D-dimer, and ferritin in patients without recovery (P<0.001), (Figure 3).

Determination of sensitivity and specificity of laboratory biomarkers

For evaluating these variables as predictors of COVID-19 mortality, ROC analysis was performed (Figure 4). Among laboratory biomarkers, CRP level, LDH level, WBC count, absolute lymphocyte count, 25-OH vitamin D level, IL-6 level, D-dimer level, and ferritin level were selected for ROC analysis. All selected markers had high area under curve (AUC) values (Table 4). Optimal cutoffs based on the Youden index were calculated to classify the patients into two categories of high or low risk for fatal outcomes based on these variables. LDH and D-dimer levels had the highest sensitivity (1.00) and specificity (0.88). In the final model of multiple binary logistic regression (after adjusting age, CRP, albumin, WBC, D-dimer, LDH, and 25-OH vitamin D), IL-6 and ferritin remained in the model (Table 5). When the two biomarkers were combined, the resulting regression model was as follows (Equation 1):

1. Login (P)=-3.787+0.004 IL-6+0.002 ferritin

Discussion

Although population-based studies have described changes in laboratory biomarkers in COVID-19 patients, risk classification of laboratory biomarkers to predict clinical deterioration of the disease is very important for treatment. We have introduced a predictive model for classifying COVID-19 patients into low-risk and high-risk groups with a recent COVID-19 diagnosis, applying

Table 4. Variables analyzed as potential biomarkers for mortality

Variables	ROC Curve		Risk Factor Cutoff Characterization			P
	AUC	95% CI	Cutoff	Sensitivity	Specificity	
CRP level (mg/dL)	0.70	0.57-0.83	39.5	88.2	56.6	=0.01
LDH level (U/L)	0.80	0.69-0.90	649	100	56.6	<0.0001
WBC count (/µL)	0.73	0.59-0.86	5400	60.4	82.4	=0.005
Lymphocyte count (/µL)	0.78	0.64-0.91	1200	84.9	70.6	=0.001
25 OH-vitamin D level (ng/mL)	0.74	0.59-0.88	14.1	66.0	76.5	=0.003
IL-6 level (pg/mL)	0.92	0.85-0.99	147	94.1	81.1	<0.0001
D-Dimer level (ng/mL)	0.82	0.72-0.93	5894	58.8	88.7	<0.0001
Ferritin level (ng/mL)	0.85	0.75-0.94	452	94.1	66.0	<0.0001

ROC: Receiver operating characteristic; AUC: Area under curve.

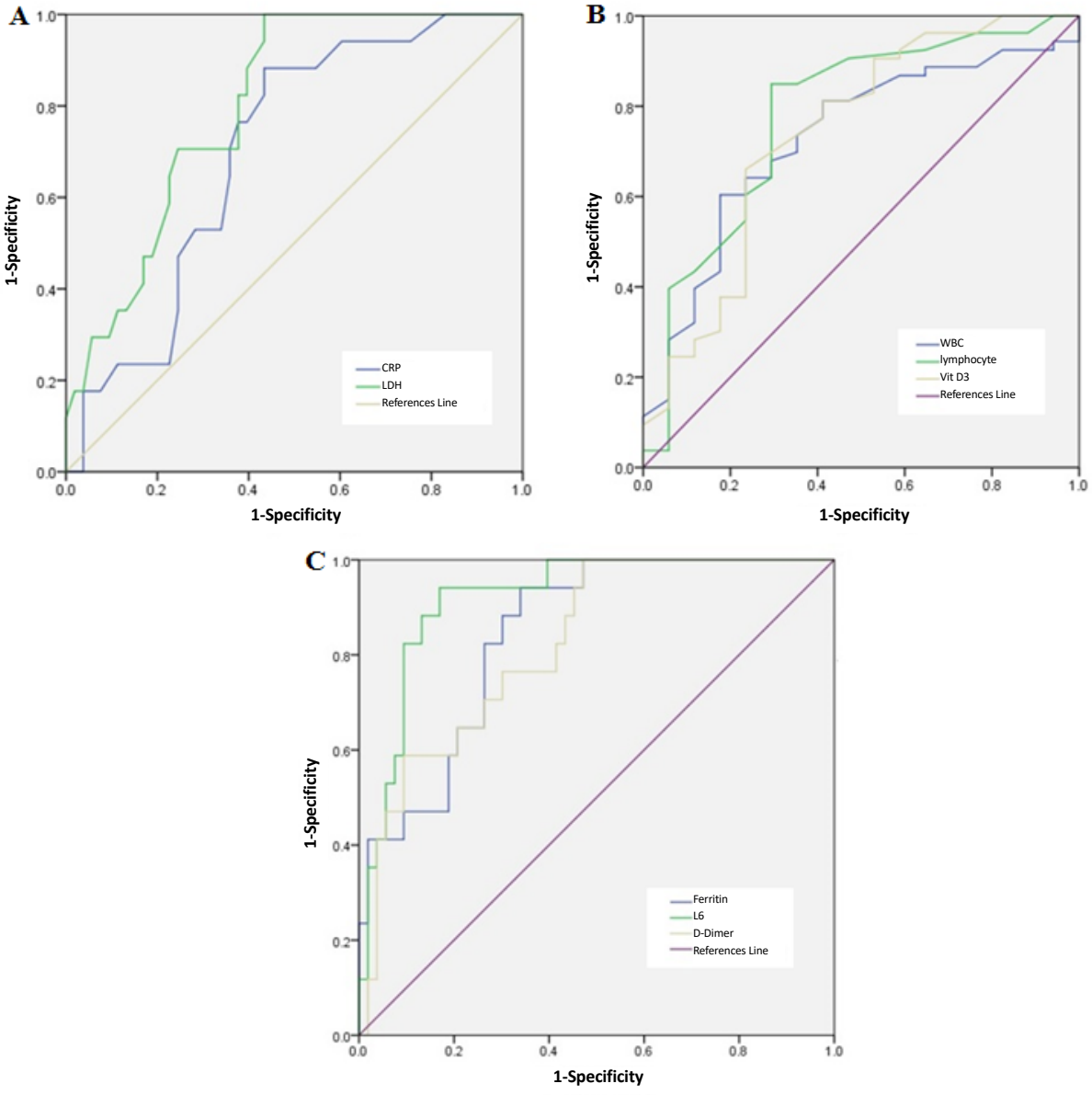

Figure 4. ROC curve analyses of laboratory biomarkers in patients with different outcomes

A) ROC curves for CRP and LDH, B) ROC curves for WBC, lymphocyte count, and Vitamin D3, C) ROC curves for ferritin, interleukin (IL)-6, and D-Dimer

for early, easy-to-assess biomarkers based on their probability of death. Patients with poor prognosis had lower

Table 5. Final multiple binary logistic regression model for predicting mortality

Variables	SE	OR	95% CI	P
Ferritin	0.001	1.002	1-1.005	0.05
IL6	0.002	1.004	1.001-1.008	0.01

The data are presented after adjusting for age, C-reactive protein, albumin, white blood cells, D-dimer, lactate dehydrogenase, and 25-OH vitamin D.

Research in Molecular Medicine

lymphocyte count, albumin, and 25-OH vitamin D but higher CRP and LDH amounts on admission. Moreover, it was observed that patients with poor prognosis have also increased levels of IL-6, D-dimer, and ferritin five days after hospitalization. To reduce confounding factors in this study, we chose individuals aged 20 to 40 years who had no history of diabetes, hypertension, coronary heart disease, chronic obstructive pulmonary disease, chronic hepatitis, and malignancy.

A typical characteristic of coronavirus infection is lymphopenia [18, 19], which may be caused by lymphocyte apoptosis directly induced by coronaviruses [20, 21]. Several studies have indicated that in coronavirus infection, there is a decrease in white blood cell count and a reduction of absolute lymphocyte count in both CD4-T cells and CD8-T cells, as well as natural killer cells [22]. In the present study, absolute lymphocyte count in the patient group was also reduced compared to the healthy subjects, and in patient groups, a greater decrease in counts of these cells was observed among those who died of COVID-19. Based on the area under the receiver operating characteristic curve (AUROCC) analysis, the absolute lymphocyte count could be a potential biomarker for mortality (AUROCC=0.78).

A progressive inflammatory reaction was proposed as a potential mechanism for COVID-19 infection [23]. In the innate immune response, CRP is considered a downstream acute phase protein [24]. In particular, levels are affected by IL-6 levels; hence, its cellular transcription can be regarded as a direct result of IL-6 signaling [25]. Hence, serum CRP level is usually used as a biomarker of inflammation. CRP and LDH are valuable markers widely considered in clinical decision-making. The present study showed that CRP and LDH are highly sensitive to mortality prediction (0.88 and 1.00, respectively).

Patients with severe forms of COVID-19 are suspected to develop hyperactivation of the immune system associated with unregulated secretion of inflammatory cytokines [26]. In this process, IL-6 may play a major role [27]. Some studies have reported that inhibiting the IL-6/IL-6R signaling pathway by the humanized anti-IL-6 receptor mAb tocilizumab is beneficial for treating patients [28, 29]. In our study, the IL-6 level was raised in non-survivors, indicating that the circulating IL-6 amount is highly connected to COVID-19 severity [12, 26, 30], which has also been further demonstrated in a recent meta-analysis [31]. However, some articles reported that IL-6 levels were comparable between mild and severe COVID-19 patients [23, 32]. It should be considered that IL-6 levels are related to an increased death risk in cases entering the ARDS phase rather than COVID-19. In our study, we set up IL-6 level measurement in COVID-19 cases as a laboratory marker for inflammation and a guide for administering tocilizumab. It is considerable that blocking IL-1 using anakinra is also helpful in decreasing invasive mechanical ventilation and, as a result, the mortality rate among cases with severe COVID-19 [33], suggesting the role of pro-inflammatory cytokines in COVID-19 disease and its consequences.

In our cohort, the probability of death was significantly increased when the D-dimer level was higher than 5894 ng/mL. We decided to use a D-dimer threshold of 5894 ng/mL as having a higher specificity for detecting thrombotic complication patients in which an increased D-dimer was correlated with an elevated risk of patient mortality. Similar to Zhou et al. [12] and in contrast to Laguna-Goya [8], our study confirmed the predictive value of D-dimer level for survival of patients (Table 4). Zhou et al. argued that the D-dimer level in 81% of non-survivors was higher than 1000 ng/mL, while in Laguna-Goya study, only 13% of non-survivors D-dimer level was above the mentioned threshold.

In the present cohort, the patients with COVID-19 who admitted to the hospitalist service indicated that the highest risk of death happened when the IL-6 level was greater than 147 pg/mL, ferritin level greater than 452 ng/mL, LDH level greater than 649 U/L, CRP level greater than 39.5 mg/dL, and 25 OH-vitamin D level lower than 14.1 ng/mL. These findings indicate that the inflammatory markers are associated with critically ill patients requiring ventilator care or dying. Considering all these issues, these markers may predict clinical course. Further studies are warranted on the timing and frequency of the mentioned inflammatory biomarkers and the value of trending these markers as they are associated with clinical outcomes.

After adjusting age, CRP, albumin, WBC, D-dimer, LDH, and 25-OH vitamin D, the final model of multiple binary logistic regression included only two variables: IL-6 and ferritin. They can be accessed conveniently in clinical laboratories and hospitals and acquired quickly. Indeed, every one-unit increase in IL-6 and ferritin has a 0.2% (95% CI, 0.1%, 0.8%) and 0.1% (95% CI, 0%, 0.5%) increase in odds of death, respectively.

The present study has some limitations. First, this is a study of a single center with a small sample size. Second, the study aimed to investigate the risk factors of prognosis, but the poor prognosis group had a small sample size. Third, we missed asymptomatic, and our cohort study did not include COVID-19 subjects who had mild cases treated at home; hence, the study represents a more

severe population of COVID-19. Finally, these patients' treatment was clinically driven and not a unified standard.

Considering all these limitations, it can be concluded that these biomarkers can help clinicians identify hospitalized COVID-19 patients at risk of clinical deterioration. We recommend a risk model that may lead to the early detection of patients encountering a higher risk of fatal outcomes. Our finding indicated that regular checking of IL-6, D-dimer, CRP, LDH, ferritin, and absolute lymphocyte count has clinical value, specifically when these biomarkers are higher than the cutoff value mentioned. The early identification of prognostic factors and high-risk patient populations for COVID-19 mortality could lead to helping medical decisions, ensuring better monitoring of these patients, planning for hospital services (including intensive care) and reducing the mortality rate.

Conclusion

Elevated CRP, LDH, IL-6, D-dimer, ferritin, decreased absolute lymphocyte count, 25-OH vitamin D, total protein, and albumin are the risk factors associated with poor prognosis in COVID-19 patients. Our findings could help clinicians to quicken the early diagnosis of high-risk COVID-19 cases, especially in primary hospitals.

Ethical Considerations

Compliance with ethical guidelines

All individuals signed an informed consent form, and the study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (Code: IR.MAZUMS.REC.1399.7446).

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Authors contribution's

Conceptualization: Hossein Jalali, Ahmad Najafi, Lotfollah Davoodi and Mohammad Reza Mahdavi; Methodology: Hossein Jalali, Ahmad Najafi and Mohammad Reza Mahdavi; Data Analysis: Abbas Alipour; Writing–Original Draft: Ahmad Najafi; Writing–review & editing: Hossein Jalali and Mohammad Reza Mahdavi; Supervision: Mohammad Reza Mahdavi.

Conflict of interest

The authors declared no conflict of interest.

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