Original article

The Effect of Acute Phase Reactants on the Survival of COVID-19 Patients in Intensive Care

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Abstract. *Study Objectives:* The aim of this study was to investigate the relationship between the biomarkers of serum CRP, PCT, D-dimer, ferritin, and lymphocytes, and the survival of COVID-19 patients admitted to the Intensive Care Unit (ICU). *Methods:* The effect of acute phase reactants on survival were retrospectively examined in 399 patients diagnosed with COVID-19 and followed up in the ICU of Afyonkarahisar Health Sciences University Medical Faculty Hospital between 20 March 2020 and 31 December 2020. *Results:* The 399 patients included in the study comprised 273 (68.4%) males and 126 (31.6%) females with a median age of 68 years (IQR: 15 years). Mortality developed in 225 (56.4%) patients in ICU and 174 (43.6%) were discharged. In the ROC analysis applied to CRP, PCT, D-dimer, ferritin levels, and lymphocyte count, the AUC values were determined in the range of 0.389-0.635. D-dimer was the parameter with the highest AUC value. In the survival analysis according to the cutoff values determined for CRP, PCT, D-dimer, and lymphocyte count, these four parameters were determined to have an effect on survival (p = 0.038, p = 0.001, p = 0.012, and p = 0.018, respectively). Ferritin levels were found to be similar between the groups of survivors and non-survivors (p = 0.492). *Conclusion:* High serum CRP, PCT, D-dimer levels, and low lymphocyte count were determined to be associated with poor outcomes in the 399 patients admitted to the ICU with a diagnosis of severe COVID-19 pneumonia.

Key words: acute phase reactant, COVID-19, inflammatory, intensive care

Introduction

The SARS-CoV-2 virus emerged in December 2019 and caused the novel coronavirus disease 2019 (COVID-19). This infectious disease spread rapidly and was declared a global pandemic. Respiratory droplets and human to human contact are the main routes of the spread of the virus (1).

Although the majority of COVID-19 patients are asymptomatic or have a mild flu-like disease, a small proportion may develop severe pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure, which may result in death (2).

There is evidence that severe COVID-19 patients showing hyper inflammation have high serum C-reactive protein (CRP), procalcitonin (PCT), D-dimer, and hyperferritinemia. The first reports of the pathological characteristics of patients who have died from severe SARS-CoV-2 infection have shown that proinflammatory cytokine levels were increased at a high rate (3).

Huang et al suggested that white blood cell count, neutrophil count, lymphocyte count, and D-dimer,

albumin, and PCT levels are risk factors in COVID-19 patients for admittance to ICU (4).

The aim of this study was to investigate the relationship between the biomarkers of serum CRP, PCT, D-dimer, ferritin, and lymphocytes, and the survival of COVID-19 patients admitted to the Intensive Care Unit.

Materials and Methods

This retrospective study was conducted in the COVID-19 ICU of Afyonkarahisar Health Sciences University Medical Faculty Hospital between 20 March 2020 and 31 December 2020. Approval for the study was granted by the Clinical Research Ethics Committee of the University Medical Faculty (decision no: 125, dated: 2021).

The study included patients with severe pneumonia who had been identified as potential cases according to the COVID-19 diagnosis, treatment, and follow-up guidelines of the Turkish Ministry of Health but who had a negative RT-PCR SARS-CoV-2 test, and patients with severe pneumonia who had RT-PCR SARS-CoV-2 test positivity from nasopharyngeal or respiratory tract samples (5).

Patients admitted to ICU were evaluated in respect of demographic data, comorbidities, clinical and laboratory test findings, prognosis, and mortality. The patient data were retrieved from the hospital automated records system. The patients were separated into two groups as those who developed mortality in ICU and those who were discharged. These two groups of survivors and non-survivors were compared in respect of CRP, PCT, D-dimer, and ferritin levels and lymphocyte count at the time of diagnosis.

Statistical Analysis

Categorical variables were stated as number (n) and percentage (%). The conformity of continuous variables to normal distribution was assessed with the Shapiro Wilk test. Continuous variables showing normal distribution were stated as mean ± standard deviation (SD) values, and those not showing normal distribution as median and interquartile range (IQR) values. In the comparisons of the survivors and non-survivors, the Chi-square test was applied to categorical variables, and where necessary, Fisher's Exact test was used. In the comparisons of continuous variables between the groups, the Independent Samples t-test was applied to data showing normal distribution, otherwise, the Mann Whitney U-test was used. The predictive value of CRP, PCT, D-dimer, ferritin, and lymphocytes was examined with Receiver Operating Characteristic (ROC) curve analysis. The Youden Index was used to determine the optimal cutoff values. Kaplan-Meier (with log rank test) curves were applied in the survival analysis. To determine the parameters increasing the mortality risk, univariate and multivariate Cox regression analysis was applied. A value of $p<0.05$ was accepted as statistically significant.

Results

The 399 patients included in the study comprised 273 (68.4%) males and 126 (31.6%) females with a median age of 68 years (IQR: 15 years). Mortality developed in 225 (56.4%) patients in ICU and 174 (43.6%) were discharged. The demographic characteristics, comorbidities, symptoms on presentation, and laboratory test results of all the patients are shown in Table 1.

In the ROC analysis applied to CRP, PCT, D-dimer, ferritin levels, and lymphocyte count, the AUC values were determined in the range of 0.389-0.635. D-dimer was the parameter with the highest AUC value. The results of the ROC analysis applied to these four parameters are shown in Table 2 and Figure 1.

In the survival analysis according to the cutoff values determined for CRP, PCT, D-dimer, and lymphocyte count, these four parameters were determined to have an effect on survival ($p = 0.038$, $p = 0.001$, $p = 0.012$, and $p = 0.018$, respectively). Ferritin levels were found to be similar between the groups of survivors and non-survivors ($p = 0.492$). The survival analysis for the CRP, PCT, D-dimer, and ferritin levels, and lymphocyte count of the groups is shown in Figure 2.

In the multivariate Cox regression analysis, only an elevated PCT level was determined as an independent risk factor for mortality. The results of the univariate and multivariate Cox regression analyses

Table 1. The demographic characteristics, comorbidities symptoms on presentation, and laboratory test results of the patient groups.

	Non-survivor (n = 225)	Survivor (n = 174)	*p*
Demographics and clinical characteristics Age (interval)	70-12	66-17.2	< **0.001***
Male sex	70.2-158	66.1-115	0.387**
Hypertension	51.1-115	40.2-70	**0.034****
Diabetes mellitus (n-%)	34.7-78	28.2-49	0.194**
Coronary artery disease (n-%)	28.4-64	20.1-35	0.062**
Asthma-Chronic obstructive lung disease (n-%)	20.4-46	19-33	0.800**
Chronic kidney failure (n-%)	9.8-22	5.7-10	0.193**
Malignancy (n-%)	14.7-33	8-14	**0.043****
Fever	7.1-16	14.4-25	**0.020***
Shortness of breath	99.1-223	100-174	0.507*
Dry cough	58.2-131	63.2-110	0.353*
Fatigue	23.6-53	20.7-36	0.545*
Myalgia	5.3-12	8.6-15	0.230*
Headache	4.4-10	5.7-10	**0.646***
Laboratory findings			
PaO_2	60.8-11.9	63.2-16.3	**0.001***
PaO_2/FiO_2	110-22	120-31.2	< **0.001***
Lactate (mg/dL)	19-10	17-8	**0.007***
WBC (103/uL)	9320-6460	8670-5402	0.315*
Lymphocyte (103/uL)	620-520	790-580	< **0.001***
Hemoglobin (g/dL)	12.6-3	12.65-2.2	0.709*
Platelet count (103/uL)	196-118	226-113	**0.007***
C-reactive protein. (mg/dL)	11.6-11.3	10.7-10.1	**0.005***
Procalcitonin (ng/mL)	0.338-0.91	0.182-0.35	< **0.001***
d-Dimer (µg/mL)	1.2-2.8	0.83-1.2	< **0.001***
Ferritin (ng/mL)	756-1007	656.5-833.9	0.073*
Fibrinogen (mg/dL)	511.57 ± 159.1	524.11 ± 142.1	0.063***
Alanine aminotransferase (ALT) (U/L)	24-24.5	25-31	0.149*
Creatinine (mg/dL)	1.01-0.64	0.9-0.39	**0.014***
Albumin (g/dL)	3.14-0.52	3.19-0.55	0.924*
Sedimentasyon (mm/h)	60.23±26.4	64.2±26.1	0.734***
*Mann Whitney U test, **Fisher's exact test, ***Independent samples t test			

Table 2. The ROC analysis results and optimal cutoff values for CRP, PCT, D-dimer, ferritin levels, and lymphocyte count

	Cut-off	Sensitivity	Specificity	AUC	*p* - value	%95 GA
C-reactive protein(mg/dL)	13.55	%45.8	%69	0.582	**0.005**	0.526-0.638
Procalcitonin (ng/mL)	0.219	%59.6	%62.6	0.624	< **0.001**	0.569-0.679
d-Dimer (µg/mL)	0.95	%64.4	%57.5	0.635	< **0.001**	0.580-0.689
Ferritin (ng/mL)	863	%45.3	%66.1	0.552	0.073	0.496-0.609
Lymphocyte (103/uL)	795	%37	%51.1	0.389	< **0.001**	0.333-0.444

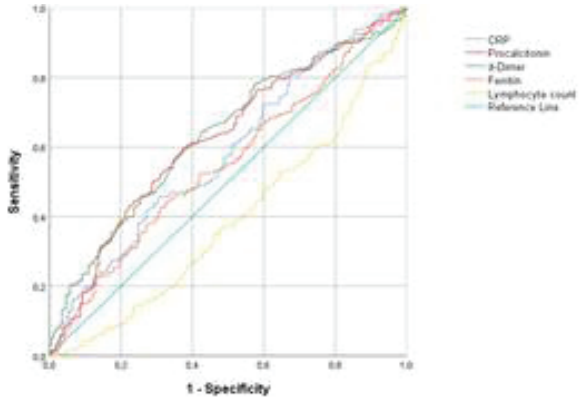

Figure. 1. The ROC analysis results for CRP, PCT, D-dimer, ferritin levels, and lymphocyte count

performed to examine the parameters affecting mortality are shown in Table 3.

Discussion and Conclusion

In-hospital mortality rates of severe COVID-19 patients have been reported at rates of up to 40%, and the rate in this study was even higher at 56.4% (6). When evaluating a patient with COVID-19 infection, biomarkers may be of use to the clinician in respect of starting treatment and close monitoring. Siddiqi and Mehra stated that in the hyper inflammation phase of COVID-19, there is a significant increase

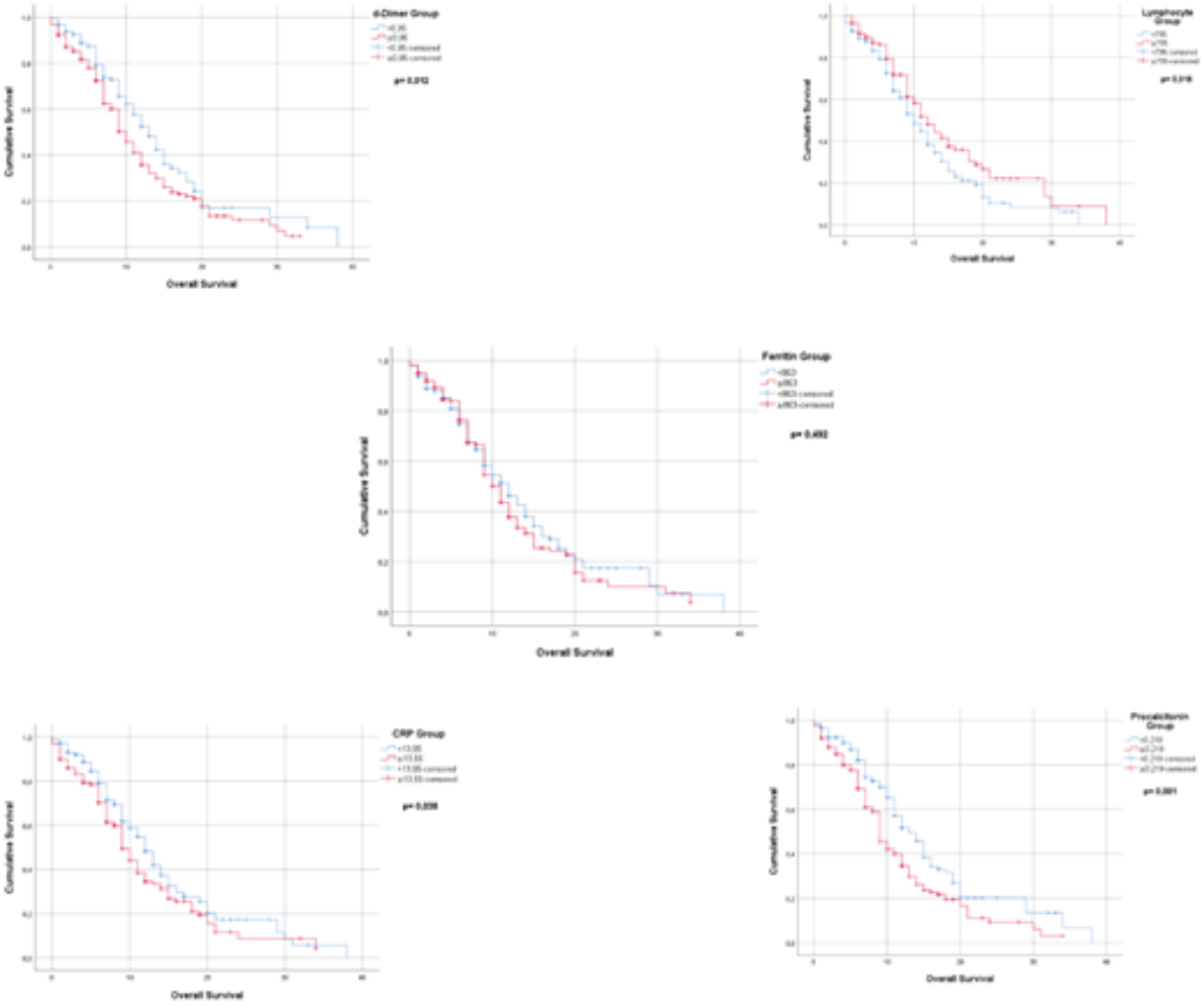

Figure. 2. Survival analysis according to the optimal cutoff values

Table 3. The results of the univariate and multivariate Cox regression analyses performed to examine the parameters affecting mortality

	Univariate			Multivariate		
Variables	HR	%95 GA	p	HR	%95 GA	p
Age > 68	1.13	0.87-1.48	0.346			
Hypertension	1.04	0.80-1.35	0.745			
Diabetes mellitus	1.18	0.89-1.55	0.240			
Coronary artery disease	1.05	0.92-1.20	0.437			
Chronic kidney failure	1.62	1.04-2.53	0.030	1.44	0.91-2.26	0.115
Malignancy	1.51	1.04-2.20	0.027	1.41	0.96-2.08	0.077
Fever	0.59	0.35-0.99	0.047	0.62	0.37-1.04	0.070
CRP>13,55	1.30	1.01-1.70	0.046	1.18	0.89-1.56	0.240
Procalcitonin>0,219	1.54	1.18-2.02	0.001	1.41	1.06-1.88	0.017
d-Dimer>0,95	1.4	1.06-1.84	0.017	1.20	0.90-1.59	0.212
Ferritin>863	1.09	0.84-1.42	0.509			
Lymphocyte >795	0.73	0.55-0.96	0.024	0.79	0.59-1.04	0.096
HR, hazard ratio.						

in biomarkers and inflammatory cytokines such as interleukin (IL)-2, IL-6, IL-7, granulocyte colony stimulating factor, macrophage inflammatory protein 1-α(MIP-1α), tumor necrosis factor-alpha (TNF-α), CRP, ferritin, PCT, and D-dimer. It has been reported that uncontrolled hyper inflammation can lead to cardiopulmonary collapse and multiple organ failure (7).

CRP, which is induced by IL-6 in the liver, is a non-specific acute phase protein, which is a sensitive biomarker of inflammation, infection, and tissue damage (8). In normal conditions, the level of CRP expression is generally low, but it increases rapidly and significantly during an acute inflammatory response (9).

In a meta-analysis that included 13 studies of COVID-19 patients, Huang et al reported that a high serum CRP level was correlated with severe COVID-19 infection and the need for ICU, but there was no relationship with mortality. According to the ROC analysis in that meta-analysis, serum CRP level ≥10mg/L showed diagnostic value for poor outcomes of COVID-19 infection (mortality, ARDS, ICU, and severe COVID-19) with the sensitivity of 51% and the specificity of 88% (10).

Liu et al investigated the relationship between serum CRP level and COVID-19 infection and reported that patients with serum CRP >41.8 mg/L were at greater risk of developing severe COVID-19 (11). Li et al showed that the serum CRP level could be used in the monitoring of the progression and recovery of patients with COVID-19 infection (12).

In the current study, the serum CRP cutoff value of 13.55 mg/dL had 45.8% sensitivity and 69% specificity for the determination of the prognosis of COVID-19 infection. As there are differences in the reported cutoff values of serum CRP levels for the determination of COVID-19 infection severity, different results have been seen. Together with the serum CRP levels for the prediction of prognosis of COVID-19 infection, it must not be forgotten that serum CRP levels can be affected by various factors such as age, gender, smoking status, weight, lipid levels, blood pressure, and liver damage (13).

Procalcitonin (PCT) is a glycoprotein without hormone activity and is a precursor of calcitonin hormone (14). Although it is still a matter of debate whether PCT can differentiate bacterial and viral pneumonia, it has been found to reduce side-effects and the period of use of antibiotics in acute respiratory tract infections, and improve survival rates (15).

In a meta-analysis, a high serum PCT level was shown to be correlated with severe COVID-19 infection and mortality, and in the ROC analysis, a serum

PCT level of 0.5mg/L was shown to have a diagnostic value with 88% sensitivity and 68% specificity (10).

Liu et al showed that according to Cox analysis, serum IL-6 ($p < 0.001$), CRP ($p < 0.001$) and PCT (p=0.002) levels could be used as independent risk factors for the prediction of the severity of COVID-19 infection (11).

In the ROC analysis in the current study of patients with a mortal course of COVID-19 infection, a serum PCT cutoff value of 0.219 ng/mL had a diagnostic value with 59.6% sensitivity and 62.6% specificity. Consistent with the literature, the PCT level of > 0.219 ng/mL was determined in the multivariate Cox regression analysis to be an independent risk factor for mortality ($p = 0.017$). High serum PCT levels in severe COVID-19 patients suggest that there could be accompanying bacterial infections. High serum PCT levels could be of guidance in the antibiotic treatment of bacterial superinfection, but there is a need for further studies on this point.

D-dimer is a fibrin destruction product that occurs as a result of activation of the fibrinolytic system. The D-dimer level is often increased in COVID-19 patients (36%-43%) and has been reported to be associated with serious complications and mortality (16). In a retrospective study of 191 COVID-19 patients, serum D-dimer levels > 1.0 μg/mL ($p = 0.0033$) were found to be related to increased mortality. It was reported that D-dimer levels of ≥ 2.0μg/mL during hospitalization were the optimum level for the prediction of in-hospital COVID-19-related mortality (17).

In a study by Gao et al which evaluated the sensitivity and specificity of different variables used in the determination of severe COVID-19 patients, the AUC values of IL-6 and D-dimer were 0.795 and 0.750, respectively, in ROC analysis, and it was reported that the IL-6 and D-dimer levels could be used to predict the severity of COVID-19 (18).

In the current study, the D-dimer cutoff value for the determination of COVID-19 infection prognosis was 0.95 μg/mL, with 64.4% sensitivity and 57.5% specificity. In the ROC analysis applied to the CRP, PCT, D-dimer, and ferritin levels, and lymphocyte count, D-dimer had the highest AUC value (0.635). This was consistent with the literature and suggested that it could be used for the prediction of disease severity.

The serum ferritin level, which is used in the diagnosis of iron deficiency anemia, is an acute phase reactant that may be increased in viral infections. In a retrospective clinical series by Ruan et al, the data predicting COVID-19 mortality were analyzed, and it was reported that the levels of IL-6, ferritin, and CRP were higher in non-survivors than survivors (19).

Huang et al examined acute phase reactant levels in severe COVID-19 infection in a meta-analysis and reported that the serum ferritin level was independently associated with severe COVID-19, ARDS, and mortality (10).

In the current study, the CRP, PCT, D-dimer, and lymphocyte count were determined to have an effect on survival ($p = 0.038$, $p = 0.001$, $p = 0.012$, and $p = 0.018$, respectively), while the ferritin level was not seen to affect survival ($p = 0.492$). As there were insufficient facilities, serum IL-6 levels were not examined, which could be said to be a limitation of the study.

Several studies have reported that there could be a relationship between lymphopenia and the severity of COVID-19 infection. Tan et al showed that lymphopenia could be an effective and reliable marker for disease severity and hospitalization in COVID-19 patients (20). Yang et al reported lymphopenia at the rate of 80% in severe COVID-19 patients, while Chen et al found lymphopenia at the rate of only 25% in patients with mild COVID-19 infection (21, 2).

In the current study, the cutoff value for lymphocytes in the determination of the prognosis of COVID-19 infection was determined to be 795 ng/mL with 37% sensitivity and 51.1% specificity according to the ROC analysis.

The aim of this study was to investigate the effect of the biomarkers of serum CRP, PCT, D-dimer, ferritin, and lymphocytes on the survival of patients admitted to the Intensive Care Unit with COVID-19 pneumonia. The results demonstrated a relationship between the laboratory biomarkers of high serum CRP, PCT, D-dimer levels, and low lymphocyte count, and poor outcomes of COVID-19. To be able to save the lives of patients with these biomarkers, more aggressive treatments must be applied at an earlier stage. The validity of PCT as an independent risk factor for the prediction of mortality in COVID-19 patients must be further investigated in studies with larger samples.

Conflict of Interest: The authors declare that there is no conflict of interest in this manuscript.

Ethical Approval and Consent to participate: The study was conducted following the Declaration of Helsinki, and patients gave their written consent. Approval for the study was granted by the Clinical Research Ethics Committee of Afyonkarahisar University of Health Sciences (decision no:125, dated:2021)

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