European Review for Medical and Pharmacological Sciences 2023; 27: 8952-8961

Inflammatory markers and neopterin levels in relation to mild COVID-19

A. KARABULUT[1], M. ŞAHIN[2]

[1]Department of Internal Medicine, Faculty of Medicine, Hitit University, Çorum, Turkey
[2]Department of Medical Biochemistry, Faculty of Medicine, Hitit University, Çorum, Turkey

Abstract. – OBJECTIVE: The immunopathology of acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection involves an excessive inflammatory response. Approximately 80% of patients with coronavirus disease 2019 (COVID-19) have a mild illness, 20% require hospitalization, and approximately 5% require intensive care. Neopterin is a macrophage activation marker produced by monocytes and macrophages following activation by interferon-gamma (IFN-γ). It is crucial to determine neopterin levels and evaluate them together with inflammatory, coagulation, and biochemical markers in moderate/mild SARS-CoV-2 infection.

PATIENTS AND METHODS: The present study compared plasma neopterin and inflammatory as well as biochemical markers of 50 patients with COVID-19 and 38 healthy volunteers without COVID-19.

RESULTS: The data of 38 participants did not show statistically significant differences in serum neopterin levels between the mild/moderate COVID-19 and control groups (p=0.259). White blood cell (WBC), neutrophil, lymphocyte, platelet (PLT), hemoglobin (HGB), procalcitonin (PCT), ferritin, prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR) and lymphocyte CRP ratio (LCR) values were significantly different between the study groups (p<0.001, p=0.001, p=0.001, p<0.001, p=0.014, p<0.001, p=0.001, p<0.001, p=0.004, p<0.001, p<0.001, respectively). According to the ROC analysis, the WBC, PT, Na, and LCR values exceeding the cutoff values may be predictive of COVID-19.

CONCLUSIONS: Although there were no significant differences in serum neopterin levels between the groups, there were high values in patients with severe SARS-CoV-2 infection in previous studies and these values were maintained for a long time. The present study found that neopterin levels were not elevated in mild/moderate COVID-19 patients.

Key Words:
COVID-19, Inflammatory markers, Neopterin, SARS-CoV-2.

Introduction

A pneumonia epidemic caused by a new type of coronavirus broke out in Wuhan, China in December 2019[1]. The disease rapidly spread throughout China. Afterwards, many countries reported that new cases of coronavirus pneumonia began to appear[2]. The World Health Organization (WHO) declared the virus "severe acute respiratory syndrome (SARS)-coronavirus (CoV-2)", and the disease was named "coronavirus disease 2019" (COVID-19) in February 2020[3,4]. Because of the global spread of the disease, COVID-19 was officially declared a pandemic by the WHO on March 11, 2020[5]. SARS and Middle East respiratory syndrome (MERS) are diseases caused by coronaviruses and SARS caused a pandemic between 2002 and 2003. China and Hong Kong were the most affected countries by the pandemic. The MERS pandemic emerged in Saudi Arabia and was caused by MERS-CoV[6,7].

Approximately 80% of patients with COVID-19 have a mild illness, 20% require hospitalization and approximately 5% require intensive care[8]. Globally, the disease's mortality rate was approximately 3.4% and reached as high as 4.3% in Wuhan, where COVID-19 originated[9]. It is already known that COVID-19 has a poor prognosis in elderly male patients and patients with comorbidities such as cardiovascular disease, diabetes, or chronic obstructive pulmonary disease (COPD)[10-12]. It is also known that some laboratory parameters, such as lymphopenia, D-dimer, ferritin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and elevated C-reactive protein (CRP), were reported as prognostic factors in different studies[8,13,14]. However, there are still uncertainties regarding the prediction of the poor prognosis of COVID-19 cases.

The present study evaluated neopterin alone or CRP, lymphocytes, the lymphocyte CRP ratio (LCR), the neutrophil-lymphocyte ratio (NLR), ferritin, white blood cells, and procalcitonin

Corresponding Author: Alpaslan Karabulut, MD; e-mail: dralpaslan_78@hotmail.com

(PCT) in hospitalized patients, especially patients with moderate/mild COVID-19. This study aimed to measure the predictive values of SARS-CoV-2 infection in combination with other inflammatory markers, such as SARS-CoV-2, and some biochemical parameters.

Neopterin (1', 2', 3'-D-erythro-trihydroxypropylpterin) is classified in the pteridine family and is involved in various oxidation-reduction reactions in the body. It is formed *in vivo* from guanosine triphosphate (GTP). This reaction is catalyzed by the enzyme GTP-cyclohydrolase-I (GCH I), particularly in activated monocytes, macrophages, dendritic cells, and endothelial cells upon stimulation by interferon-gamma (IFN-γ). Neopterin is released by macrophages as a response to cytokines that are released by T lymphocytes and natural killer cells. This shows that neopterin plays a role in the activation of cell-mediated immunity, which is strictly associated with hyperinflammation[15]. For this reason, targeting neopterin in SARS-CoV-2 infection may be critical for the early prediction of disease progression and the timely assessment of infected individuals. Neopterin levels fell steadily during COVID-19 in moderate cases, but in severe cases, this decline was slower. Neopterin, which was investigated in a few studies[16] among individuals infected with SARS-CoV-2, can be used to predict the severity of COVID-19.

Patients and Methods

This case-control study employed 88 patients in total, including 50 patients diagnosed with COVID-19 based on the WHO criteria and whose respiratory samples (i.e., nasopharyngeal swab or invasive respiratory sample) were positive for SARS-CoV-2 RT-PCR and hospitalized in the Internal Medicine/COVID-19 ward, and 38 gender- and age-matched healthy controls who applied to the internal medicine outpatient clinic. Patients (n=50) infected with SARS-CoV-2 were hospitalized in the Internal Medicine/COVID-19 ward of Hitit University Erol Olçok Training and Research Hospital between April 15 and July 15, 2021, due to symptomatic SARS-CoV-2 viral infection. The blood samples of the patients with a positive SARS-CoV-2 PCR test during their admission to the Internal Medicine/COVID-19 ward were collected in a serum separation tube. The sera for each individual in the case and control groups were stored frozen at -80°C until use. Pregnancy, end-stage chronic kidney disease, chronic inflammatory diseases, critically ill patients hospitalized in intensive care units, and patients with cancer were excluded from the study. Routine laboratory results such as demographic-clinical characteristics, comorbidities, blood cell count (CBC), liver and kidney function tests, venous blood gas, prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), D-dimer, ferritin, CRP, and PCT were recorded in the study form for all patients during their admission to the Internal Medicine/COVID-19 ward.

Neopterin Measurement

The remaining blood samples, after routine examinations, were centrifuged at 3,500 rpm for 10 minutes and their serums were separated and stored in an Eppendorf tube at -80°C. The kits and serum samples were kept at room temperature (+25°C) for 30 minutes to check enzyme levels. Neopterin levels were studied in the sera of the participants using ELISA and were measured in nmol/L using Human Neopterin ELISA kits (Bioassay Technology Laboratory, Shanghai, China; Catalog No: E3155Hu).

Ethics Committee

This is a prospective case-control study that adhered to the Declaration of Helsinki. Ethical approval was obtained from the Hitit University Clinical Study Ethics Committee (2021/435), and written informed consent forms were obtained from all participants.

Statistical Analysis

SPSS version 22.0, (IBM Corp., Armonk, NY, USA) software was used for data analysis. The normality of the data distribution was determined using the Kolmogorov-Smirnov and Shapiro-Wilks tests. Descriptive statistics of categorical data were given as frequencies (n) and percentages (%). For normally distributed continuous data, descriptive statistics were reported as mean±standard deviation (SD) and median (min-max) for nonnormally distributed data. Proportion comparisons between study groups were performed using the Chi-square test or Fisher's exact test, depending on the sample size in the crosstab cells. Student's *t*-test for normally distributed data and the Mann-Whitney U test for nonnormally distributed data were used to compare continuous data between two independent groups. A receiver operating characteristic (ROC) analysis was conducted using blood values to distinguish the COVID-19 group from the control group. ROC curves and area under the curve (AUC) and 95%

confidence intervals of this area were calculated for all blood values found to be significant by basic statistical tests. The AUC values obtained in the analyses were 0.9-1: excellent, 0.8-0.9: good, 0.7-0.8: fair, 0.6-0.7: poor, and 0.5-0.6 very poor. The best cutoff point for the WBC, PT, Na, and LCR values with AUC values greater than 0.75 in ROC analysis was determined using the Youden index (highest sensitivity and specificity). The success of the cutoff points was evaluated by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) values. The statistical significance level was set at $p<0.05$.

Results

The study analyzed the data of 88 patients; 56.8% (n=50) of the patients were in the COVID-19 group, and 43.2% (n=38) were in the control group. Additionally, 45.5% (n=40) of the patients were female and 54.5% (n=48) were male. The mean age of the patients was 41.05±15.13 (min-max: 18-80), the mean age of the COVID-19 group was 42.84±16.67, and the mean age of the control group was 38.71±12.65. The mean age of the study groups was not significantly different ($p>0.05$). The mean length of stay of COVID-19 patients was 10.82±3.71 (min-max: 2-26 days). The male and female ratios were statistically similar between the study groups ($p>0.05$). A total of 42% (n=21) were female and 58% (n=29) were male in the COVID-19 group, while 50% (n=19) were female and 50% (n=19) were male in the control group. Comorbidity rates were different at a statistically significant level between the study groups ($p=0.009$). Neopterin values were not significantly different between comorbidity groups ($p=0.655$). The mean neopterin level in the group without comorbidities was 3.41±1.83 [median (min-max): 2.81 (1.25-7.74)] and the mean neopterin level was 3.31±1.88 in the group with comorbidities [median (min-max): 2.14 (1.36-6.67)]. There were no statistically significant correlations between the neopterin levels and the length of stay in COVID-19 patients (r=-0.095, $p=0.510$). Table I presents the comparison of sociodemographic characteristics and comorbidity status among the study groups.

Table II shows the comparison of the study groups in terms of neopterin, WBC, neutrophil, neutrophil %, lymphocyte, lymphocyte %, PLT, HGB, PCT, CRP, fibrinogen, D-dimer, ferritin, PT, aPTT, INR, AST, ALT, NLR, PLR, and LCR values. WBC, neutrophil, lymphocyte, PLT, HGB, PCT, ferritin, PT, aPTT, INR and LCR values were significantly different between the study groups ($p<0.001$, $p=0.001$, $p=0.001$, $p<0.001$, $p=0.014$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.004$, $p<0.001$, $p<0.001$, respectively, Table III). WBC, neutrophil, lymphocyte, PLT, HGB, and LCR values were significantly lower in the COVID-19 group (Table II). PCT, ferritin, PT, aPTT, and INR values were significantly higher in the COVID-19 group (Table II). The other parameters were not significantly different between the study groups ($p>0.05$).

Table III shows the comparison of glucose, urea, creatinine, Na, K, pH, HCO_3, pCO_2, pO_2, saturation %, and lactate values among the study groups. Urea, creatinine, Na, K, pO_2, saturation %, and lactate values were significantly different between the study groups ($p=0.009$, $p<0.001$, $p<0.001$, $p=0.008$, $p=0.023$, $p=0.027$, $p=0.003$, respectively, Table III). Na values were significantly lower in the COVID-19 group (Table III). Urea, creatinine, K, pO_2, saturation %, and lactate values were significantly higher in the COVID-19 group (Table III). Other variables were not significantly different between the study groups ($p>0.05$). Figure 1 indicates box plots showing the distribution of WBC, PT, Na, and LCR values between the COVID-19 and control groups. ROC analysis was performed for all blood values found to be significant as a result of basic statistical tests among the study groups. AUC values obtained in ROC analysis are shown in the last column of Table II and Table III.

WBC, PT, Na, and LCR values with AUC values of 0.75 and above were significant in the differentiation of COVID-19 as a result of the ROC analysis [AUC=0.771 (0.671-0.871), AUC=0.763 (0.664-0.862), AUC=0.797 (0.702-0.891), AUC=0.764 (0.664-0.864), respectively] (Table IV). Table IV presents the sensitivity, selectivity, and positive-negative predictive values calculated using the cutoff values determined using the Youden index to determine the success of the prediction of COVID-19. The ROC curve is shown in Figure 2. The cutoff point for the WBC value was 5.52. The classification success for this cutoff point was as follows: 60% for sensitivity and 89.4% for selectivity (Table IV). The cutoff point for the PT value was 9.795. The classification success for this cutoff point was as follows: 58% for sensitivity and 89.5% for selectivity (Table IV). The cutoff point for the Na value was found to be 139.5. The classification

Table I. Comparison of sociodemographic and comorbidity status of patients between COVID-19 and control groups.

		Control (n=38)	COVID-19 (n=50)	*p*-value
Age (year)		38.71±12.65	42.84±16.67	0.190[c]
Gender	Female	19 (50%)	21 (42%)	0.455[a]
	Male	19 (50%)	29 (58%)	
Comorbidity	No	38 (100%)	41 (82%)	0.009[b]
	Yes	0 (0%)	9 (18%)	

[a]Chi square test with n (%). [b]Fisher's exact test with n (%). [c]Student's *t*-test with mean± SD.

Table II. Comparison of blood values of patients between COVID-19 and control groups.

	Control (n=38)	COVID-19 (n=50)	*p*-values	AUC (95% CI)
Neopterin (nmol/L)	2.61 (0.76-9.10) (3.24±2.31)	2.81 (1.25-7.74) (3.39±1.82)	0.259[d]	-
WBC (10^9/L)	7.26 (3.66-12.91) (7.194±1.82)	5.11 (3.39-11.83) (5.61±1.672)	<0.001[d]	0.771(0.671-0.871)
Neutrophil (10^9/L)	4.21 (1.98-7.85) (4.16±1.29)	2.87 (1.27-7.39) (3.23±1.46)	0.001[d]	0.717 (0.609-0.825)
Neutrophil (%)	58.06±7.21	56.71±13.43	0.548[c]	-
Lymphocyte (10^9/L)	2.24±0.67	1.74±0.65	0.001[c]	0.697 (0.588-0.806)
Lymphocyte (%)	31.71±7.198	32.21±11.37	0.799[c]	-
PLT (10^9/L)	259.5±51.12	210.2±66.45	<0.001[c]	0.735 (0.631-0.840)
HGB (g/dL)	14.42±1.71	13.44±1.92	0.014[c]	0.649 (0.534-0.764)
PCT (ng/ml)	0.035 (0.01-0.10) (0.039±0.02)	0.053 (0.02-1.02) (0.094±0.146)	<0.001[d]	0.745 (0.643-0.847)
CRP (mg/l)	3.14 (3.14-10.40) (3.51±1.22)	4.05 (3.13-124) (19.11±31.75)	0.175[d]	-
Fibrinojen (mg/dl)	308 (3.10-479) (304±71.06)	312.5 (171-733) (338.1±107.9)	0.441[d]	-
D-dimer (ug/ml)	0.192 (0.10-2.40) (0.301±0.407)	0.257 (0.10-9) (0.704±1.489)	0.201[d]	-
Ferritin (ng/ml)	56.5 (3-360) (77.71±69.11)	112.4 (6.48-2,521) (266.3±494.3)	<0.001[d]	0.737 (0.632-0.841)
PT (sn)	9.27 (8.5-10.4) (9.28±0.47)	9.95 (8.66-26.3) (10.89±3.04)	<0.001[d]	0.763 (0.664-0.862)
aPTT (sn)	24.95 (20.1-29.5) (24.99±2.121)	27.45 (16.8-55.8) (27.59±5.584)	0.004[d]	0.678 (0.566-0.791)
INR	1.02 (0.94-1.16) (1.03±0.05)	1.09 (0.97-2.78) (1.19±0.31)	<0.001[d]	0.738 (0.635-0.841)
AST (U/L)	19.5 (13-47) (21.05±7.097)	19.5 (10-120) (24.84±16.71)	0.403[d]	-
ALT (U/L)	19.5 (10-83) (22.81±13.77)	20.5 (10-156) (29.08±23.66)	0.284[d]	-
NLR (%)	1.89 (0.85-3.81) (1.97±0.72)	1.86 (0.54-9.12) (2.26±1.70)	0.840[d]	-
PLR (%)	120.94 (54.22-239.05) (123.24±34.89)	118.65 (58.33-460.34) (137.03±75.61)	0.893[d]	-
LCR (%)	0.676 (0.101-1.245) (0.680±0.224)	0.401 (0.005-0.990) (0.389±0.300)	<0.001[d]	0.764(0.664-0.864)

[c]Student's *t*-test with mean± SD. [d]Mann-Whitney U test with median (min-max). CRP: C-reactive protein, WBC: White blood cell, PLT: Platelet count, PCT: Procalcitonin, PT: Prothrombin time, Na: Sodium, HGB: Hemoglobin, aPTT: Activated partial thromboplastin time, INR: International normalized ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, LCR: Lymphocyte to CRP ratio.

Table III. Comparison of blood values of patients between COVID-19 and control groups.

	Control (n=38)	COVID-19 (n=50)	*p*-values	AUC (95% CI)
Glucose (mg/dl)	93.5 (62-152) (97±16.15)	92 (69-260) (102.9±32.46)	0.953[d]	-
Urea (mg/dl)	23.5 (13-37) (25.05±6.693)	30 (14-120) (34.98±20.55)	0.009[d]	0.662 (0.550-0.775)
Creatinine (mg/dl)	0.7 (0.4-1.1) (0.7±0.167)	0.9 (0.4-2) (0.948±0.361)	<0.001[d]	0.739 (0.636-0.841)
Na (mmol/L)	141 (136-145) (141.2±2.04)	138.5 (131-143) (138.4±2.72)	<0.001[d]	0.797 (0.702-0.891)
K (mmol/L)	4.4 (3.9-4.9) (4.39±0.27)	4.2 (3-24) (4.57±2.82)	0.008[d]	0.666 (0.553-0.778)
pH	7.36±0.035	7.38±0.036	0.065[c]	-
HCO_3 (mEq/L)	25.9 (19.7-30.3) (25.95±2.51)	25.2 (15.1-31.1) (25.17±3.07)	0.211[d]	-
pCO_2 (mmHg)	44.07±7.147	42.44±5.794	0.241[c]	-
pO_2 (mmHg)	29.9 (11.6-62.2) (29.52±11.65)	33.05 (16.1-62.9) (35.28±11.69)	0.023[d]	0.642 (0.525-0.759)
Saturation %	52.05±16.22	60.38±17.88	0.027[c]	0.621 (0.504-0.738)
Lactate (mmol/L)	1.71 (1.01-3.83) (1.81±0.62)	2.15 (0.92-3.82) (2.19±0.62)	0.003[d]	0.687 (0.571-0.803)

[c]Student's *t*-test with mean±SD. [d]Mann-Whitney U test with median (min-max). Na: sodium, K: potassium, HCO_3: bicarbonate, pCO_2: partial pressure of carbon dioxide, pO_2: partial pressure of oxygen.

Table IV. ROC (receiver operating characteristic) analysis results, sensitivity, specificity, and positive-negative predictive values for the success of blood values in distinguishing COVID-19 patients.

	WBC	PT	Na	LCR
AUC (95% CI)	0.771 (0.671-0.871)	0.763 (0.664-0.862)	0.797 (0.702-0.891)	0.764 (0.664-0.864)
Cutoff	5.52	9.795	139.5	0.5029
Sensitivity	60% (45.2-73.2)	58% (43.2-71.5)	62% (47.1-75)	68% (53.1-80)
Specificity	89.4% (74.2-96.5)	89.4% (74.2-96.5)	89.4% (74.2-96.5)	84.2%(68-93.4)
PPV (True Positive)	88.2% (71.6-96.1)	87.8% (70.8-96)	88.5% (72.3-96.2)	85% (69.4-93.7)
NPV (True Negative)	62.9% (48.7-75.3)	61.8% (47.7-74.2)	64.1% (49.7-76.5)	66.6% (51.4-79.1)

AUC: area under the ROC curve, CI: confidence interval; PPV: positive predictive values, NPV: negative predictive values; WBC: White blood cell, PT: Prothrombin time, Na: Sodium, LCR: Lymphocyte to CRP ratio.

success for this cutoff point was as follows: 62% for sensitivity and 89.4% for selectivity (Table IV). The cutoff point for the LCR value was 0.5029. The classification success for this cutoff point was as follows: 68% for sensitivity and 84.2% for selectivity (Table IV).

Discussion

In the present study, we investigated whether there was a significant difference in serum neopterin levels between the study groups (mild/moderate COVID-19 and control groups). Serum

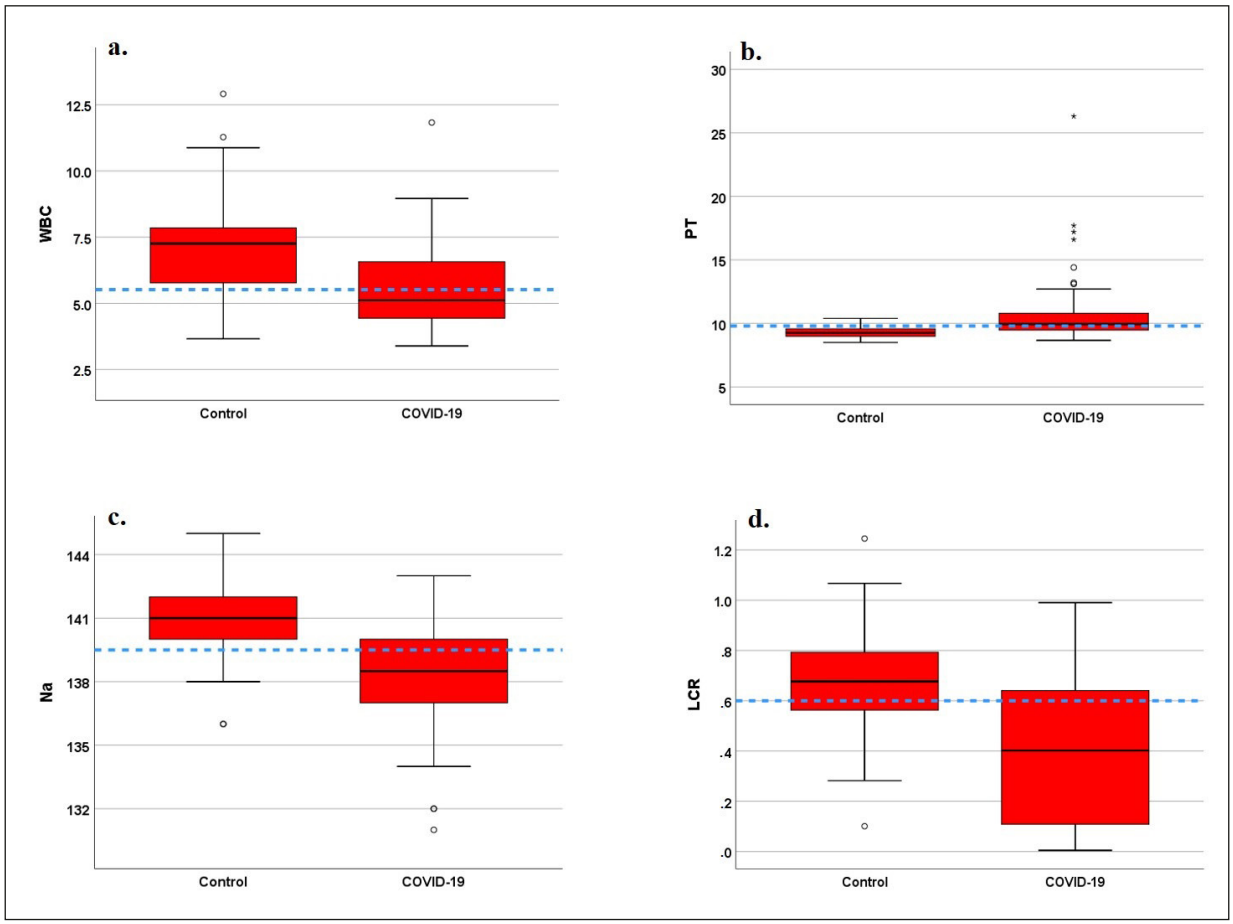

Figure 1. **a**, Box-plot plot showing the distribution of WBC values between COVID-19 and control groups. **b**, Box-plot plot showing the distribution of PT values between COVID-19 and control groups. **c**, Box-plot plot showing the distribution of Na values between COVID-19 and control groups. **d**, Box plot showing the distribution of LCR values between the COVID-19 and control groups. *: Extreme outliers. °: Mild outliers.

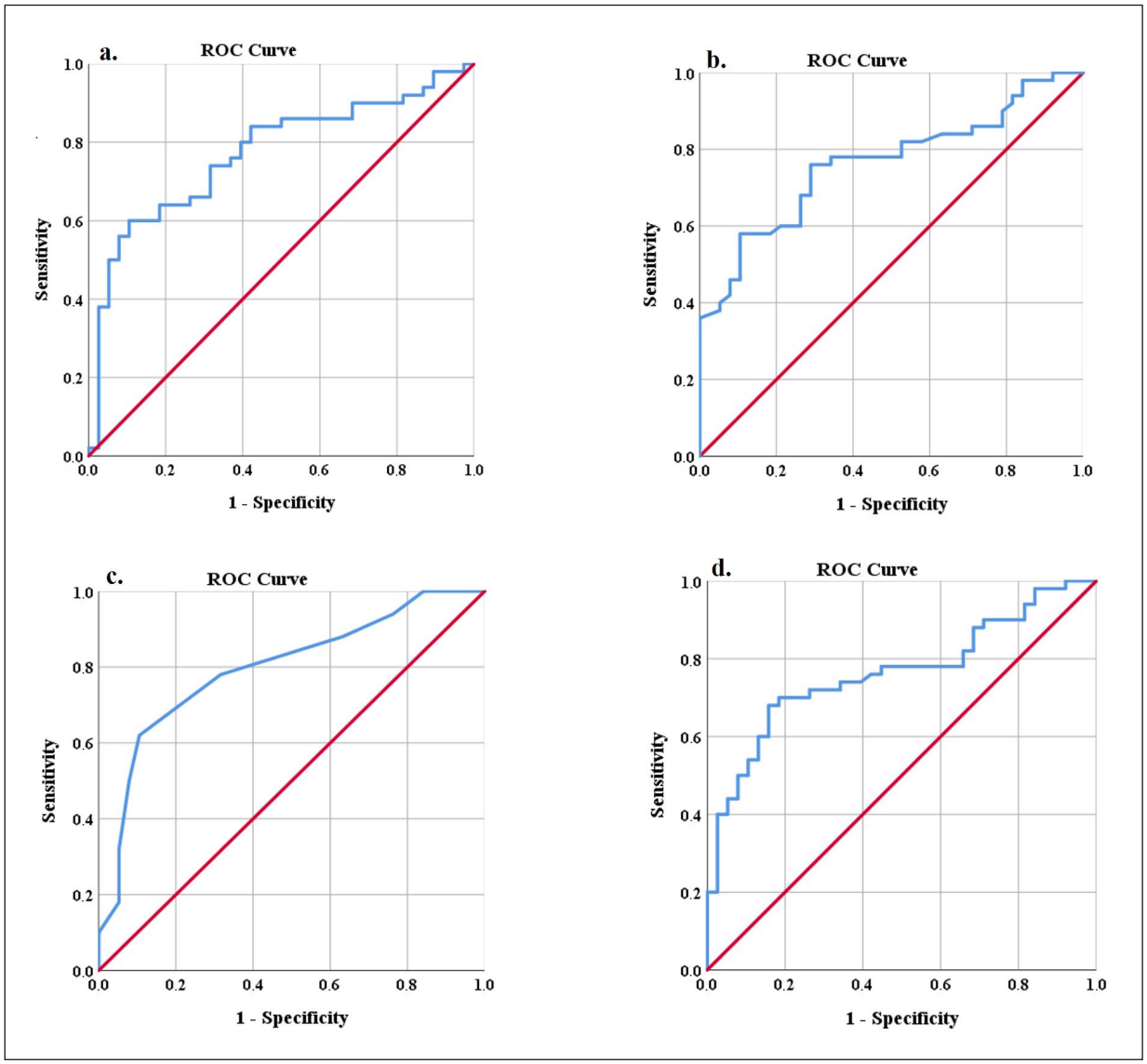

Figure 2. **a**, The area under the ROC curve for COVID-19 – control discrimination by WBC values. **b**, The area under the ROC curve for COVID-19 – control discrimination by PT values. **c**, The area under the ROC curve for COVID-19 – control discrimination by Na values. **d**, The area under the ROC curve for COVID-19 – control discrimination by LCR values

neopterin levels measured during hospitalization in patients with moderate/mild COVID-19 were not significantly different from those in healthy volunteers ($p>0.05$) (Table II). Neopterin values were not significantly different between comorbidity groups ($p=0.655$). WBC, neutrophil, lymphocyte, PCT, ferritin, and LCR among the inflammatory markers measured between the two groups were significantly different ($p<0.001$) (Table II).

The immune response to SARS-CoV-2 in symptomatic patients is usually hyperactive, and this condition is characterized by an excessive inflammatory response in the lung, especially in patients with acute respiratory distress syndrome (ARDS). This is mediated to a large extent by the release of high levels of proinflammatory cytokines such as Interlukine-6 (IL-6), Tumour Necrosis Factor alpha (TNF-α), and Interlukine-1 (IL-1), and the increase in the expression of cell adhesion molecules[17]. Then, the uptake of neutrophils and inflammatory monocytes into the lung increases[18].

In a study conducted during the 2002 SARS epidemic, serum neopterin levels were elevated in SARS patients on the day of symptom onset. Neopterin levels were five times higher in patients with acute SARS infection than in recovered individuals and four times higher than in healthy controls.

High neopterin concentration was associated with a longer febrile period and, therefore, a more severe course of the disease in SARS patients[19].

COVID-19 is associated with the activation of the inflammatory response, and only a few studies[16,20] have evaluated the level of neopterin in patients with COVID-19. The study conducted by Robertson et al16 found that the mean neopterin concentration was two times higher in severe patients than in patients with mild symptoms. Neopterin levels decreased gradually in both mild and severe patients, but high neopterin levels in severe cases lasted longer. They also predicted in their study that neopterin serum levels might be an independent prognostic factor for determining the severity of COVID-19. The prospective study conducted by Ozger et al[20] on 134 patients, showed that severe COVID-19 patients had higher neopterin levels when compared to mild cases, with a fourfold increase in comparison to the healthy group. In this respect, a high neopterin serum level may be a useful biomarker for recognizing COVID-19 patients at high risk of adverse outcomes such as intensive care unit admission or mechanical ventilation. This study further suggested that neopterin might be a diagnostic biomarker in viral infections of the lower respiratory tract, as the serum level of neopterin increased by more than 10 nmol/L in 96% of the patients. In a retrospective study by Bellmann-Weiler et al[21] with 115 hospitalized severe COVID-19 patients, those with a neopterin level >45 nmol/l had a fourfold higher risk of mortality than patients with a neopterin level ≤45 nmol/l. The intensive care unit (ICU) hospitalization rate was 14-fold higher, and the need for mechanical ventilation was 16-fold higher. In another study[22], the level of neopterin in the cerebrospinal fluid was high in patients with severe SARS-CoV-2 infection with neurological disorders. For this reason, the significant elevation of the neopterin biomarker might be associated with the neurological symptoms of COVID-19. Based on the above-mentioned evidence, high neopterin levels might help in the early recognition of patients who may end up with adverse conditions, such as mechanical ventilation and ICU admission. This evidence also indicates that neopterin can be used as a prognostic marker to monitor the progression of COVID-19.

Some clinical studies[16,20,23,24] proved that mild cases have lower neopterin levels when compared to severe cases, and neopterin levels in mild cases return to normal levels more quickly. In particular, patients with high neopterin levels at initial admission need close follow-up and similarly early referral to noninvasive ventilation to reduce the potential for complications and mechanical ventilation and ICU admission, which is associated with long-term care[21]. Karacaer et al[25] conducted a study on patients with moderate and severe COVID-19 and reported no significant difference in serum neopterin levels. The present study found no statistically significant differences in neopterin levels at the time of hospitalization compared with the control group. There might also have been a decrease in neopterin levels sometime after the onset of symptoms. For this reason, the results obtained in the present study made us predict that the patients would not result in ICU hospitalization, need for mechanical ventilation, or death. Indeed, none of the 50 COVID-19 patients in the present study had any of these conditions, and eventually, the patients were discharged with full recovery.

The present study also evaluated inflammatory markers (WBC, neutrophil, lymphocyte, PCT, CRP, fibrinogen, ferritin, NLR, PLR, and LCR). WBC, neutrophil, lymphocyte, PCT, ferritin, and LCR values were significantly different among the study groups (Table II). The sensitivity calculated to determine the success of WBC and LCR values [AUC=0.771 (0.671-0.871), AUC=0.764 (0.664-0.864, respectively) with AUC values of 0.75 and above] that were found to be significant in the differentiation of COVID-19 as a result of the ROC analysis in the prediction of COVID-19, and selectivity and positive-negative predictive values are given in Table IV. The ROC curve is shown in Figure 2.

The severity of SARS-CoV-2 infection is related to changes in WBC levels[26]. There was a small increase in WBC count in those with acute COVID-19, while there was a clinically significant increase in individuals who died. In conclusion, a significant increase in WBCs in patients with severe disease may indicate clinical deterioration and a higher risk of poor prognosis[27]. Lymphocytes are considered to be essential for eliminating cells infected with the SARS virus. Survival may be associated with the capacity to regenerate lymphocytes destroyed by this virus[28]. The significantly lower WBC count in the present study might be associated with a severe decrease in the lymphocyte count. This also leads us to think that it may have a predictive value for moderate/mild COVID-19 cases.

LCR, one of the inflammatory markers, has been studied in a limited number of studies in the literature. The most important article on this

topic is a meta-analysis on the role of LCR in predicting disease severity, which reported that LCR was significantly lower in SARS-COV-2 patients with severe disease[29]. The present study found that the LCR has a predictive value in COVID-19. We measured this rate at a lower level in COVID-19 patients than in the healthy control group.

Table III presents the comparison of glucose, urea, creatinine, Na, K, pH, HCO_3, pCO_2, pO_2, saturation %, and lactate values among the study groups. Urea, creatinine, Na, K, pO_2, saturation %, and lactate values were significantly different between the groups (p=0.009, p<0.001, p<0.001, p=0.008, p=0.023, p=0.027, p=0.003) (Table III). Na values were significantly lower in the COVID-19 group (Table III). Table IV shows the sensitivity, selectivity, and positive-negative predictive values of Na [AUC=0.797 (0.702-0.891)], which were calculated in the prediction of COVID-19 and found to be significant in the differentiation of COVID-19 as a result of the ROC analysis and whose AUC value was 0.75 and above. The ROC curve is shown in Figure 2.

Hyponatremia is common among COVID-19 patients and can sometimes only present with symptoms and clinical manifestations secondary to this electrolytic imbalance. The diagnosis of hyponatremia at the time of admission of a patient during the COVID-19 pandemic should raise suspicion of a possible SARS-CoV-2 infection. The causes of hyponatremia in these patients are varied, and it is very important to determine the precise etiology of this electrolytic disorder because therapeutic management differs depending on its pathophysiological mechanism[30]. Serum sodium imbalance is associated with severe illness, poor clinical outcome(s), and increased in-hospital mortality in COVID-19 patients and can be considered an adverse prognostic factor among COVID-19 patients[31,32]. The present study indicated that Na has a predictive value in COVID-19. We measured the Na value at a lower level in COVID-19 patients than in the healthy controls.

COVID-19 increases the risk of venous and arterial thrombosis[33]. The current study also evaluated coagulation tests (D-dimer, aPTT, PT and INR). Among these, aPTT, PT and INR were significantly different between the groups (Table II). Based on the ROC analysis, the AUC value of the PT was significant in the differentiation of COVID-19 and in those whose AUC values were 0.75 and above [0.763 (0.664-0.862)]. Table IV shows the sensitivity, selectivity, and positive-negative predictive values calculated to determine the success of the prediction of COVID-19. The ROC curve is shown in Figure 2.

Wang et al[14] showed that PT prolongation was detected in 58% of COVID-19 patients. A retrospective and multicenter study[13] that included 191 COVID-19 patients found PT to be one of the factors associated with nonsurvival. In a study conducted by Tang et al[34] on COVID-19 patients, those who lost their lives at the time of admission to the hospital had a significantly longer PT than those who survived. In the present study, PT had a predictive value in COVID-19. We measured the PT value to be longer in COVID-19 patients than in the healthy control group.

Conclusions

The present study compared inflammation markers, coagulation values, biochemical markers and neopterin in plasma between healthy people with and without COVID-19. Serum neopterin levels showed no statistically significant differences between mild/moderate COVID-19 and healthy people without COVID-19. Serum neopterin levels did not increase in mild COVID-19. Further studies are needed to determine its clinical significance and prognostic value in severe COVID-19.

Additionally, in the present study, we anticipate that the inflammatory markers LCR and WBC are significantly lower, biochemical values and sodium are significantly lower, and coagulation factors and PT are significantly longer in moderate/mild COVID-19 patients.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Informed Consent

Written informed consent was obtained from the study participants.

Funding

The authors received no financial support for this study.

Ethics Approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics Committee Approval was obtained from the Hitit University Clinical Study Ethics Committee (2021/435).

Acknowledgments
We are grateful to the healthcare professionals in the internal medicine clinic for their efforts.

Authors' Contributions
Alpaslan Karabulut: concept, design, supervision, data collection, literature search, writing manuscript, critical review, analysis and interpretation, and resources.
Mustafa Şahin: concept, design, data collection, analysis, literature review, manuscript writing, critical review, resources, materials and editing.

Data Availability
Data information can be obtained from the author upon request.

ORCID ID
Alpaslan Karabulut: 0000-0003-2893-692X
Mustafa Şahin: 0000-0001-6073-563X

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