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RESEARCH ARTICLE

The predictive role of biomarkers for mortality in COVID-19 patients

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ABSTRACT

Many biomarkers are used in addition to radiologic examinations to determine the severity of COVID-19. This study aims to determine WBC, neutrophil, lymphocyte, platelet, D-dimer, CRP, AST, ALT, LDH, PT, APTT, INR, urea, creatinine, lactate, and ferritin levels in COVID-19 patients and the effect of their changes on mortality rate. The study was conducted between 11 March 2020 and 31 August 2020 (during the COVID-19 pandemic). A total of 502 patients older than 18 years who presented with suspected COVID-19 were included in the study. Of these 502 patients who applied to the hospital, 229(45.6%) were male and 273(54%) were female. 301(60%) patients were diagnosed with COVID-19 through computed tomography and PCR tests. 201(40%) patients with negative test results constituted the control group. Patients with positive test results 48.2% (n=145) were men, and 51.8% (n=156) were women. The median age of the patients was 51±25 years. The patients tested positive for COVID-19 were divided into three groups as outpatients (26.9%), inpatients (68.8%), and intensive care unit patients (4.3%). The mortality rate of the patients followed via the patient follow-up system after 30 days was determined as 2.7%. The biomarker values of patients examined in this study tested negative and positive for COVID-19 were compared. In the study, D-dimer, ferritin, Lactate, AST, ALT, LDH, Urea, Creatinine, APTT, and INR levels were found to be higher in the positive tested patients than the negative ones. In the study, it was concluded that neutrophil, lymphocyte, CRP, and ferritin ratios should also be followed in the follow-up phase of the disease. It is important that additional measures should be taken in cases when these biomarkers increase by following the values of the patients who started taking treatment. Also, the ratio of biomarkers is crucial in determining whether the treatment has been effective or not.

Keywords: COVID-19; biomarkers; ferritin; CRP; NLR.

INTRODUCTION

The new coronavirus, SARS-CoV-2 (COVID-19), was first detected in China, in Hubei Wuhan, in December 2019. COVID-19, the coronavirus 2019 disease, spread throughout the world and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. COVID-19 has killed many people worldwide as a result of pneumonia and other complications. Patients with COVID-19 experience many symptoms, such as fever, shortness of breath, cough, sputum, nausea, vomiting, diarrhea, myalgia, loss of taste and smell, headache, anorexia, and weakness. In addition, pneumonia can lead to death by causing acute respiratory distress syndrome. Many biomarkers are used in addition to radiologic examinations to determine the severity of the disease. These biomarkers are useful in predicting the prognosis of patients and in managing treatment processes (Montrief *et al*., 2020).

Some biomarkers are commonly used in COVID-19. These are the levels of white blood cell (WBC), neutrophil, lymphocyte, platelet, D-dimer, c-reactive protein (CRP), procalcitonin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), lactate dehydrogenase (LDH), urea, creatinine, ferritin, lactate, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and International Normalized Rate (INR). In the resource materials, different parameters were investigated in different studies, and it was reported that it may affect mortality in the patients with COVID-19. Accordingly, it was aimed to determine WBC, neutrophil, lymphocyte, platelet, D-dimer, CRP, AST, ALT, LDH, PT, APTT, INR, urea, creatinine, lactate, and ferritin levels in the patients with COVID-19 and to determine the effect of their change on mortality rate. Therefore, it will make possible an evaluation with a more holistic approach, and it will be easier to follow the health status of the patients according to the obtained data.

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MATERIALS AND METHODS

Ethics committee approval

Ethical approval was obtained from Ordu University Clinical Research Ethics Committee (Date: 10 December 2021, Decision Number: 2020/256).

Patients

The population of this study consists of patients admitted to Ordu University Department of Emergency Medicine between March 11 and August 31, 2020. The patients admitted with fever and respiratory-related symptoms and considered infected with COVID-19 by imaging methods were evaluated. Computed tomography (CT) and PCR analysis were carried out on 502 patients admitted to the emergency service. In addition, blood samples were routinely taken from each patient for WBC, neutrophil, lymphocyte, platelet, D-dimer, CRP, AST, ALT, LDH, PT, APTT, INR, urea, creatinine, lactate, and ferritin tests. Afterward, the routine treatment protocol of the Ministry of Health was applied to the patients who were positive for COVID-19. The inpatients were followed up for 30 days, and their health status was evaluated.

Data Collection

The date of admission to the emergency department for each patient enrolled in the study, as well as the age, gender, comorbidities (e.g., hypertension, diabetes mellitus (DM), chronic obstructive pulmonary disease/asthma (COPD), coronary artery disease (CAD), and SARS-CoV-2 PCR analysis obtained in the emergency department, were documented. In addition, WBC, neutrophil, lymphocyte, platelet, D-dimer, CRP, AST, ALT, LDH, PT, APTT, INR, urea, creatinine, lactate, and ferritin, patient discharge details (including outpatient unit follow-up visits), the hospitalization status (inpatient, intensive care unit), and the 30-day mortality status were retrospectively collected using a hospital computerized database.

Laboratory Analysis

Blood tests obtained from peripheral venous blood samples of the patients were retrospectively analyzed. Complete blood count analysis (e.g., WBC, neutrophil, lymphocyte) of the patients was studied using the Sysmex XN-1000 device. Biochemical parameters (e.g., the CRP) of the patients were studied with the Roche Cobas c 701 device. Ferritin tests of the patients were conducted with the Roche Cobas e 801 device. Combined throat-nose swabs were taken from the patients in the emergency service, and SARS-CoV-2 PCR analyses were performed in the Ordu Clinical Microbiology Laboratory.

Ethical Considerations

Before the study, ethical approval was obtained from the Ordu University Local Clinical Research Ethics Committee (Date: 10 December 2020; Decision number 2020/256). Because the study was conducted retrospectively, the need for informed consent forms from patients was waived. The study was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

The data analysis was carried out by using SPSS v26 software (IBM Inc., Chicago, IL, USA). The data was given in numbers and percentages. The relationship between the types of the comorbidities and treatment settings or the patient mortality was investigated by using the Chi-square t-test. The relationship between biomarkers and mortality was examined by using the Mann-Whitney U test. Before the analysis, the data were tested for normality by using the Kolmogorov-Smirnov test and for variance homogeneity by using the Levene test. The statistical evaluation was considered significant when $P<0.05$.

RESULTS

Of 502 patients admitted to the emergency service, 229 (45.6%) were male, and 273 (54%) were female. Of these, 301 (60%) were found to be tested positive for COVID-19 through computed tomography (CT) and PCR analyses. 201 (40%) patients who were tested negative for COVID-19 constituted the control group.

The patients tested positive for COVID-19 48.2% (n = 145) were men, and 51.8% (n = 156) were women. The median age of the patients was 51 ± 25 years. The patients tested positive for COVID-19 were divided into three groups as outpatients (26.9%), inpatients (68.8%), and intensive care unit patients (4.3%). The mortality rate of the patients followed via the patient follow-up system after 30 days was determined as 2.7%.

We did not find a statistically significant relationship between the gender of the patients and the follow-up status. In addition, we did not find a significant relationship between gender and 30-day mortality. We found a statistically significant relationship between the presence of DM ($p<0.05$), HT ($p<0.001$), COPD ($p<0.001$), or CAD ($p<0.001$) and 30-day mortality. In addition, the presence of HT ($p<0.01$), COPD ($p<0.01$), or CAD ($p<0.05$) was significantly related to the follow-up status (Table 1). The biomarker values of patients examined in this study tested negative and positive for COVID-19 are given in Table 2. According to the Mann Whitney-U test, there is a significant difference between the patients tested positive and the patients tested negative in the ratios of D-dimer, CRP, ferritin, Lactate, AST, ALT, LDH, Urea, Creatinine, APTT and INR ($P<0.05$) (Table 2). According to the table, D-dimer, ferritin, Lactate, AST, ALT, LDH, Urea, Creatinine, APTT, and INR were found to be higher in the positive tested patients than the negative ones. Additionally, there is a statistically significant relationship between age ($p<0.001$), neutrophil ($p<0.05$), lymphocyte ($p<0.01$), CRP ($p<0.01$), ferritin ($p <0.05$) values, and 30-day mortality. There is a statistically significant relationship between age

Table 1. Demographic data of patients

		n	%
GENDER	Female	273	54,4
	Male	229	45,6
MORTALITY	Positive	10	2,0
	Negative	492	98,0
TREATMENT	Outdoor	266	53,0
	Inpatient	222	44,2
	Intensive care	14	2,8
DM	Positive	46	9,2
	Negative	456	90,8
HT	Positive	65	12,9
	Negative	437	87,1
CAD	Positive	32	6,4
	Negative	470	93,6
COPD	Positive	26	5,2
	Negative	476	94,8

Table 2. Biomarkers values

	SARS-CoV-2 NEGATIVE		SARS-CoV-2 POSITIVE		P
	MEDIAN	IQR	MEDIAN	IQR	
WBC	6,29	3,73	6,27	3,70	,574
NEUTROPHIL	2,11	1,90	2,76	2,76	,001
LENPHOSITE	1,59	1,15	1,48	1,00	,021
PLT	224	87	217	87	,107
D-DIMER	0,16	0,27	0,26	0,31	,000
CRP	0,46	0,94	1,36	4,92	,000
FERRITIN	63,1	105,4	130	247	,000
LAC	0,90	0,6	1,3	1	,000
AST	19	11	21	11	,002
ALT	15	13	19	16	,000
LDH	196	338	206	77	,025
BUN	9,2	2,7	11,7	10,95	,000
CRE	0,67	0,18	0,86	0,29	,000
PT	14,2	3,7	13,9	3,5	,654
PTT	29,2	6,2	29,9	6,75	,002
INR	0,95	0,77	1,6	0,65	,000

Mann Whitney – U, p<0,005 is important.

(p<0.001), WBC (p<0.001), neutrophil (p<0.01), CRP (p<0.001) values and outpatient/inpatient unit follow-up. There is a statistically significant relationship between patients' age (p <0.05), CRP (p <0.05), ferritin (p <0.01) values and inpatient unit/ICUP follow-up. A statistically significant relationship was found between the patients' age and all biomarkers except ferritin and outpatient unit/ICUP follow-up (Table 3).

DISCUSSION

The main mechanism of COVID-19 infection, the binding of the virus to membrane-bound form of angiotensin-converting enzyme 2 (ACE2) and the internalization of the complex by the host cell. The coronavirus spike (S) protein binds strongly with ACE2 and the serine protease TMPRSS2 (Tai *et al*., 2020) ACE2 and TMPRSS2 are not only found in the lungs but also in many parts of the body. They are also found in the small intestine epithelium, esophagus, liver, colon, blood vessels, heart, kidneys, ovaries, and testicles (D'Amico *et al*., 2020). Therefore, systemic effects can be seen in COVID-19 infection.

There is a need for biomarkers that can help risk stratification of patients, predict patients' prognosis early, and show response to treatment and mortality. There are biomarkers used in risk classification and disease prognosis of other diseases (Henry *et al*., 2020). COVID-19 can also cause systemic effects with the above-mentioned mechanisms. For this reason, they reported in the literature that some biomarkers can be used for this purpose. These include WBC, neutrophil, lymphocyte, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), ferritin (Du *et al*., 2020; Zhang G *et al*., 2020). In patients diagnosed with COVID-19 infection, depending on the viral effect and functional exhaustion; It has been reported that there is a decrease in the number of CD8 + T cells, cytotoxic lymphocytes (CTLs) and the total number of B cells (Cossarizza *et al*., 2020). In another study, they reported that patients with more severe COVID-19 had low lymphocyte, higher leukocyte count and higher NLR values (Chen *et al*., 2020). According to another study, COVID-19 patients with severe and fatal symptoms have been reported to have increased WBC, decreased lymphocyte, and platelet counts (Henry *et al*., 2020). In the study of Xu *et al*.

Table 3. The effect of age and biomarkers on patient follow-up and mortality

		n	%	TREATMENT				MORTALITY		
				Outdoor	Inpatient	Intensive care	p	Negative	positive	p
GENDER	Male	229	45,6%	111 48,5%	110 48,0%	8 3,5%	,153	224 97,8%	5 2,2%	,779
	Female	273	54,4%	155 56,8%	112 41,0%	6 2,2%		268 98,2%	5 1,8%	
DM	Negative	456	90,8%	247 54,2%	198 43,4%	11 2,4%	,102	449 98,5%	7 1,5%	,021
	Positive	46	9,2%	19 41,3%	24 52,2%	3 6,5%		43 93,5%	3 6,5%	
HT	Negative	437	87,1%	246 56,3%	184 42,1%	7 1,6%	,000	434 99,3%	3 0,7%	,000
	Positive	65	12,9%	20 30,8%	38 58,5%	7 10,8%		58 89,2%	7 10,8%	
CAD	Negative	470	93,6%	260 55,3%	200 42,6%	10 2,1%	,000	465 98,9%	5 1,1%	,000
	Positive	32	6,4%	6 18,8%	22 68,8%	4 12,5%		27 84,4%	5 15,6%	
COPD	Negative	476	94,8%	262 55,0%	200 42,0%	14 2,9%	,000	471 98,9%	5 1,1%	,000
	Positive	26	5,2%	4 15,4%	22 84,6%	0 0,0%		21 80,8%	5 19,2%	

Kruskal-Wallis test * <0.05 ** <0.01 *** <0.001WBC; White blood cell, NLR; neutrophil lymphocyte ratio, CRP; C-reactive protein, ICU; Intensive care unit.

(2020), it was reported that patients with a more serious clinical course or who died had a lower T lymphocyte count (Bo *et al*., 2020). In another study, it was reported that as the NLR increased, the duration of hospitalization and mortality rates increased (Liu *et al*., 2020). In this study, unlike other studies, WBC, neutrophil, lymphocyte, platelet, D-dimer, CRP, AST, ALT, LDH, PT, APTT, INR, urea, creatinine, lactate, and ferritin levels were examined in COVID-19 patients and higher rates were found compared to the ones tested negative. In addition, according to the Table 3, it was observed that there is a significant increase in age, neutrophil, lymphocyte, CRP, and ferritin ratios and a significant increase in the death rate at 30-day follow-up. It shows that neutrophil, lymphocyte, CRP, and ferritin ratios should be monitored in COVID-19 patients and necessary precautions should be taken in cases when there is an increase. In addition, similar to other studies, it was determined that mortality increases with age.

CRP is a plasma protein which produced by the liver. Various inflammatory conditions have been reported that there is a relationship between the severity of the disease and CRP levels (Gong *et al*., 2020). Zhang *et al*. reported in their study that the CRP value increased in COVID-19 patients. In addition, while the median value of CRP in COVID-19 surviving patients was 40 mg/L. This value was found as 125 mg/L in those who died. In the same study, it was reported that CRP was strongly correlated with the severity of COVID-19 and prognosis (Zhang *et al*., 2020). In addition, it was reported that with the increase of CRP in COVID-19, ARDS development and death may be seen (Terpos *et al*., 2020). In our study, it was determined that hospitalization and mortality increased as the CRP value increased. Therefore, this situation shows that CRP rates should be followed in COVID-19 patients.

Ferritin is an iron-storing protein, and its serum level reflects the normal iron level and helps in the diagnosis of iron deficiency anemia. Also, the level of circulating ferritin increases during viral infections and may be an indicator of viral replication (Li *et al*., 2020). Ferritin is a biomarker that contributes to cytokine storm by promoting the expression of multiple proinflammatory mediators (Ponti *et al*., 2020). It was reported that ferritin level increased in tables where cytokine storm was seen, such as COVID-19 (Huang *et al*., 2020). It was reported in a study that the increase in ferritin level was associated with clinical worsening of COVID-19 and even with ARDS and mortality (Wu *et al*., 2020). Another study reported that serum ferritin concentration increased in patients with high mortality risk (Dimopoulos *et al*., 2020). In our study, it was found that ferritin plays a significant role in determining the admission to intensive care unit in patients with COVID-19. In addition, a significant relationship was found between ferritin and mortality in patients with COVID-19.

As a result in the study, it was concluded that the neutrophil, lymphocyte, CRP, and ferritin ratios tested to support the diagnosis should be followed in the later phases of the disease. Additional measures should be taken in cases where these biomarkers increase by controlling the values of the patients who started taking treatment. In addition, these parameters maintain that data will be obtained about whether the treatment is effective or not thus facilitating the follow-up of the disease. Also, as age increases, the mortality rate increases, so preventive health services should be activated in these groups.

Lack of follow-up data, single blood sampling and small sample size are the limitations of our study since it is a retrospective study. To clarify the underlying mechanisms, it is necessary to confirm these findings in future studies with larger sample sizes.

Data availability

The data used in this study will be provided on demand.

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Conflict of interest statement

The author declares that they have no conflict of interests.

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