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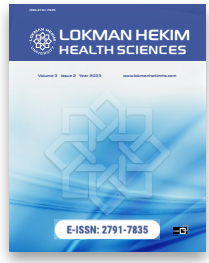

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ORIGINAL ARTICLE

Can Serum NGAL Levels be Used as an Early Prognostic Marker to Predict the Need for Intensive Care in COVID-19?

Serum NGAL Düzeyi COVID-19 Hastalarında Yoğun Bakım İhtiyacını Öngörmede Prediktif Belirteç Olabilir mi?

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Abstract

Introduction: It is important to determine which patients admitted to the hospital with COVID-19 should be hospitalized and treated. In this study, the aim was to investigate whether serum neutrophil gelatinase-associated lipocalin (NGAL) levels in the early period are related to the need for intensive care follow-up in COVID-19.

Methods: A total of 91 patients with a definite diagnosis of COVID-19 were included in the study. Patients hospitalized in the internal medicine clinic were screened cross-sectionally, and eligible patients were included in the study. Patients were divided into groups as mild/moderate pneumonia and severe pneumonia. Serum NGAL levels were measured before pulse steroid therapy. Age, gender, comorbid diseases, and 1-month survival of the patients were recorded.

Results: In patients requiring pulse steroid therapy compared to those who did not, there was a significant difference between the median serum NGAL levels. The median NGAL level showed a significant difference in patients requiring intensive care treatment compared to patients hospitalized in the inpatient service. Receiver operating characteristic curve analysis showed the relationship between serum NGAL levels and the need for treatment in the intensive care unit. It was also observed that the need for admittance to the intensive care unit was 10.9 times higher in those with a serum NGAL level below 30.29, according to the multivariate analysis. There was a significant difference between the median NGAL levels of patients who survived and those who died.

Discussion and Conclusion: This study found a significant difference between serum NGAL levels of patients who died, who required intensive care follow-up, and who needed pulse steroid therapy.

Keywords: COVID-19; Intensive care; Lipocalin-2; NGAL

It has long been known that cytokines play an important role in the immune response during viral infections.[1]Metin girmek için buraya tıklayın veya dokunun. The rapid and well-coordinated innate immune response is the first line of defense against viral infections.[2] Additional diagnosis and treatments are needed to prevent and treat existing clinical

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and pathological conditions in COVID-19 as clear treatment is yet unknown. Neutrophil gelatinase-associated lipocalin (NGAL) (human neutrophil lipocalin and lipocalin-2) is a lipoprotein involved in innate immunity.[3] Metin girmek için buraya tıklayın veya dokunun. It has been found to be negatively associated with untreated viral disease in some studies.[4] Berger et al.,[5] in an attempt to investigate the antimicrobial efficacy of NGAL, associated gene-targeted lipocalin 2 (NGAL)-deficient mice (Lcn2 –/– mice) *in vivo* with an increased susceptibility of Lcn2 –/– mice to bacterial infections. They demonstrated that neutrophils isolated from Lcn2 –/– mice showed significantly less bacteriostatic activity compared to wild-type (WT) controls, and the bacteriostatic property of WT neutrophils was eliminated by the addition of exogenous iron, indicating that the main function of lipocalin 2 in the antibacterial innate immune response is to limit iron.

Decreased lymphocyte count and low CD8+ and CD4+ T cells are an important feature of COVID-19 infection potentially also associated with dysregulation of NGAL.[6] However, COVID-19 is potentially severe in people with compromised native immune systems.[7]

Although NGAL may have a crucial role in the innate immune response to bacterial infection, no data are available on NGAL levels in patients with COVID-19. Therefore, this study investigated the relationship between serum NGAL levels and the need for intensive care.

Materials and Methods

Study Population

This prospective, cross-sectional study was conducted between September 1, 2020, and December 1, 2020.

Patients between the ages of 18 and 85 years who were hospitalized and followed up with a diagnosis of COVID-19, which was confirmed by the reverse transcription-polymerase chain reaction (RT-PCR) test, were included in the study. Patients were divided into two groups as mild/moderate pneumonia and severe pneumonia according to the COVID-19 Diagnosis Guide of the Turkish Ministry of Health. The study included a total of 91 patients. In addition to routine tests, blood samples were taken from the patients for serum NGAL level analysis. At the time of the blood draw, all patients were on the eighth day of symptom onset. In patients who received pulse steroid therapy during their hospital stay, blood samples were taken before initiation of this therapy.

The exclusion criteria were pregnancy, active smoking, chronic restrictive and obstructive pulmonary disease, acute/chronic renal failure, acute/chronic hepatic failure, malignancy, rheumatologic disease, receiving immunosuppressive therapy, recent acute myocardial infarction, history of cerebrovascular events or peripheral artery disease, alcohol and substance abuse, dementia, Parkinson's disease, and unconfirmed diagnosis of COVID-19.

Mild/moderate pneumonia was defined as having a respiratory rate of <30 min^{-1}, room air oxygen saturation (SpO_2) of >90%, and mild/moderate pneumonia findings on chest radiography or tomography. Meanwhile, severe pneumonia was defined as having tachypnea (≥30 min^{-1}), SpO_2 level at >90% in room air, and bilateral diffuse pneumonia findings on chest radiography or tomography.

The demographic (age, gender), clinical (symptoms, outcomes) characteristics, and laboratory findings of the patients were recorded from the patient files. Radiological evaluation included radiography and computed tomography.

Ethics Committee of Ankara City Hospital approval (approval no: E2-20-34, date: 02.12.2020) was obtained in addition to written and verbal consent from all patients included in the study.

The study was conducted in accordance with the Declaration of Helsinki.

Biochemical Analysis

The blood samples were collected for each participant in the morning after an overnight fast of at least 8 h. Blood was taken into a tube containing ethylenediaminetetraacetic acid for whole blood analysis. Biochemical parameters [glucose, urea, creatinine, sodium, potassium, alanine transaminase (ALT), and aspartate transaminase (AST)], ferritin, fibrinogen, interleukin-6 (IL-6), C-reactive protein (CRP), and procalcitonin were measured using standard laboratory techniques.

For NGAL level measurements, blood samples were allowed to coagulate for 30 min, and then serum and plasma levels were separated by centrifugation at 5000 rpm for 10 min. Serum samples were kept at -80°C until the day of analysis. After the sample collection was completed, the serum NGAL level was measured in the same laboratory by the same technician.

Serum NGAL Level Measurement

For the measurement of serum NGAL levels of the patients, the samples were centrifuged for 10 min after the peripheral blood samples were collected. All blood samples taken for NGAL level measurement from patients who received pulse steroids were taken before the pulse steroid treatment. The obtained serum samples were divided into Eppendorf tubes

Table 1. Treatment and prognosis distributions according to pneumonia severity

Variables	Entire population (n=91)		Degree of pneumonia				p
			Mild/moderate (n=36)		Severe (n=55)		
	n	%	n	%	n	%	
Treatment							
Favipiravir	91	100	36	100	55	100	–
Enoxaparin	91	100	36	100	55	100	–
Antibiotic	81	89	28	77.8	53	96.4	0.008
Hydroxychloroquine	47	51.6	21	58.3	26	47.3	0.302
Corticosteroids	62	68.1	12	33.3	50	90.9	<0.001
Colchicum	25	27.5	5	13.9	20	36.4	0.019
Pulse glucocorticoids	27	29.7	2	5.6	25	45.5	<0.001
Dipyridamole	5	5.5	2	5.6	3	5.5	0.661
Hospitalization time, days	15.37±12.2		9.13±4.85		19.45±13.79		–
1-month survival							0.003
Deceased	12	13.2	–	–	12	21.8	
Alive	79	86.8	36	100	43	78.2	

Numerical variables are expressed as mean±standard deviation. Categorical variables are shown as numbers (%). P<0.05 shows statistical significance.

and preserved until analysis at -80°C. NGAL levels were measured using an ELISA (BT Laboratory, Shanghai, China; Catalog Number: E1719Hu, LOT number: 202003008) 96-test kit, and samples were run according to the manufacturer's direction and quantified using a sandwich enzyme immunoassay technique. The detection range of the test is 5–600 ng/mL.

COVID-19 RT-PCR

Samples were taken from the upper respiratory tract (nose and throat) with a swab or sputum. SARS-CoV-2 RNA detection was made in the Clinical Microbiology Laboratory using Bio Speedy Bioeksen COVID-19 RT-qPCR diagnostic kits (İstanbul, Türkiye) and Coronex COVID-19 RT-qPCR diagnostic kits (Ankara, Türkiye).

Statistical Analysis

The data obtained from the data collection phase were transferred to the computer environment and analyzed using the Package for Social Sciences (SPSS-IBM Company Version 16) software. The conformity of the data to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk tests). In the evaluation of numerical data, arithmetic mean, standard deviation, median, 1st quarter, 3rd quarter, minimum, and maximum values were utilized. Frequency distributions and percentages were used to summarize categorical data. The Chi-squared (χ^2) test or Fisher's exact test was used to compare categorical data. The relationship between normally distributed numerical data and categorical data was evaluated using the t-test in independent groups, and the relationship between nonnormally distributed numerical data and categorical data was evaluated using the Mann–Whitney U test. The Kruskal–Wallis test was used to evaluate three or more groups with numerical data. A post hoc Mann–Whitney U test and Bonferroni correction were performed for pairwise comparisons between groups with significant Kruskal–Wallis test results. Correlations of nonnormally distributed numerical variables were analyzed using Spearman's correlation coefficient.

Type-1 error level was accepted as 5% for statistical significance. In the evaluation of Spearman's correlation coefficients, 0.05–0.30 was considered to be a low or insignificant correlation, 0.30–0.40 low-moderate, 0.40–0.60 moderate, 0.60–0.70 good, 0.70–0.75 very good, and 0.75–1.00 an excellent relationship. Positive correlation coefficients indicate that the variables increase and decrease together. Negative correlation coefficients indicate that while one of the variables increases the other decreases or vice versa.

The diagnostic decision-making properties of the parameters used in this study in predicting the need for hospitalization in the intensive care unit were analyzed using the receiver operating characteristic (ROC) curve analysis. In the presence of significant breakpoints, the sensitivity, specificity, positive predictive, and negative predictive values of these limits were calculated. In the evaluation of the area under the curve, the cases where type-1 error level was below 5% were interpreted statistically significant. In determining the best cutoff point with the help of the ROC

Table 2. Laboratory findings according to pneumonia severity

Variables	Entire population (n=91)	Degree of pneumonia		p
		Mild/moderate (n=36)	Severe (n=55)	
Glucose (mg/dL)	134.52±71.88	115.81±62.69	146.77±75.35	0.003
Urea (mg/dL)	51.48±23.06	38.77±14.83	59.80±23.78	<0.001
Creatinine (mg/dL)	0.93±0.25	0.89±0.25	0.96±0.24	0.088
Sodium (mEq/L)	138.57±4.42	138.97±2.46	138.30±5.34	0.129
Potassium (mEq/L)	4.21±0.57	4.28±0.49	4.17±0.61	0.432
ALT (U/L)	48.65±45.55	43.50±36.61	52.03±50.60	0.207
AST (U/L)	46.12±34.08	40.11±28.49	50.05±37.02	0.051
Leukocytes ($\times10^9$ L^{-1})	8200 (5880–10 900)	6220 (4922–7862)	9160 (7580–11 280)	<0.001
Neutrophils ($\times10^9$ L^{-1})	3889 (3670–8610)	4090 (2662–5392)	7490 (6080–9740)	<0.001
Lymphocytes ($\times10^9$ L^{-1})	1090 (630–1820)	1675 (845–2097)	910 (630–1210)	0.002
Hemoglobin (g/dL)	12.66±1.90	12.95±1.96	12.47±1.86	0.251
MCV (fL)	87.02±5.95	88.06±6.09	86.34±5.81	0.180
Thrombocytes ($\times10^9$ L^{-1})	273 000 (197 000–340 000)	256 500 (182 750–341 000)	279 000 (201 000–34 000)	0.338
INR	1.09±0.20	1.07±0.31	1.09±0.10	0.006
Fibrinogen (g/L)	4.83±1.71	3.91±1.34	5.44±1.67	<0.001
D-Dimer (mg/L)	2.24±5.61	0.88±1.08	3.12±7.06	0.017
IL-6 (pg/mL)	29.80 (11.08–78.20)	8.76 (5.65–34.30)	40.35 (20.75–89.12)	<0.001
Ferritin (µg/L)	311 (162–636)	179.5 (71–340)	464 (290–727)	<0.001
ESR (mm/h)	41 (25–65)	22.50 (5–68.5)	43 (34–65)	0.058
CRP (mg/L)	41 (11–82)	12.50 (3–41.5)	52 (34–128)	<0.001
Procalcitonin (µg/L)	0.05 (0.03–0.15)	0.03 (0.03–0.06)	0.09 (0.04–0.25)	0.001
TSH (mU/L)	0.88 (0.51–1.75)	1.21 (0.81–2.71)	0.71 (0.40–1.26)	0.005
Vitamin B12 (ng/L)	360 (276–526)	323.5 (268–392.25)	401.5 (300.75–857)	0.10
Vitamin D (ng/mL)	17.50 (11.02–27.47)	17 (12–26.80)	19.20 (10.80–28.30)	0.935

Numerical variables are shown as mean±standard deviation and median (25–75 quartiles). Categorical variables were shown as numbers (%). P<0.05 shows statistical significance. ALT: Alanine transaminase; AST: Aspartate transaminase; MCV: Mean corpuscular volume; INR: International normalized ratio; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; TSH: Thyroid stimulating hormone.

curve, the point with the highest sensitivity and selectivity was taken as the cutoff point.[8]

In multivariate analysis, independent predictors of ICU needs were examined with logistic regression analysis using possible factors identified in previous analyses. The Hosmer–Lemeshow test was used for model fit. Cases with a type-1 error level below 5% were interpreted as statistically significant.

Results

A total of 91 patients were included in the study: 36 with mild/moderate pneumonia and 55 with severe pneumonia.

The mean hospitalization time of the patients was 15.37 days. The mean length of stay (19.45 and 9.13 days, respectively; p<0.001) and mortality rate (21.8% and 0%, respectively; p<0.001) were found to be higher in patients with severe pneumonia compared to those with mild/moderate pneumonia. In the 30-day follow-up, the mortality rate was 13.2% (n=12) (Table 1).

Table 2 presents the laboratory findings for all patients. Median lymphocyte ranges in patients with severe pneumonia compared to those with mild/moderate pneumonia were found to be low [910 (min–max: 80–29 000) and 1675 (min–max: 450–3620), respectively; p=0.002]; however, levels for median leukocytes [9160 (min–max: 3420–38 800) and 6220 (min–max: 2700–15 800), respectively; p<0.001], median neutrophil [7490 (min–max: 1980–17 820) and 4090 (min–max: 1240–14 600), respectively; p<0.001], mean fibrinogen [5.44±1.67 and 3.91±1.34, respectively; p<0.001], median IL-6 [40.35 and 8.76, respectively; p<0.001], median ferritin [464 (min–max: 64–1595) and 179.50 (min–max: 13–1540), respectively; p<0.001], median CRP [52 (min–max: 0.50–347) and 12.5 (min–max: 0.50–152), respectively; p<0.001], median procalcitonin [0.09 (min–max: 0.02–6.96) and 0.03 (min–max: 0.02), respectively; p=0.001] were found to be higher.

Table 3. Association between NGAL levels, demographic characteristics, and 1-month survival

Variables	n	NGAL level					z	p
		Mean	SD	Median	Min–Max	25–75 quartiles		
Sex							-0.448	0.654
Female	51	38.65	26.05	30.92	3.39–123.33	18.85–52.91		
Male	40	42.05	30.50	32.24	2.32–129.56	24.89–53.35		
Hypertension							-0.993	0.321
Absent	41	43.44	29.85	35.51	5.09–129.56	23.60–55.13		
Present	50	37.44	26.34	30.19	2.32–123.33	20.32–52.93		
Diabetes mellitus							-0.805	0.421
Absent	53	41.96	28.68	35.51	3.39–129.56	19.91–55.76		
Present	38	37.61	27.15	30.28	2.32–123.33	21.62–43.78		
Coronary artery disease							-0.422	0.673
Absent	69	40.68	28.27	32.09	3.39–129.56	22.20–52.28		
Present	22	38.46	27.64	29.21	2.32–93.50	16.66–56.80		
Congestive heart failure							-0.655	0.512
Absent	84	40.39	27.94	32.24	2.32–129.56	21.98–52.98		
Present	7	37.22	30.57	28.09	8.04–90.53	14.02–68.61		
Thyroid disease							-0.273	0.785
Absent	82	40.59	28.80	31.99	2.32–129.56	20.44–53.64		
Present	9	36.12	19.69	28.00	20.87–80.70	22.20–46.63		
Degree of pneumonia							-1.331	0.183
Mild/moderate	36	46.18	33.20	35.90	3.39–129.56	24.29–54.99		
Severe	55	36.19	23.46	30.14	2.32–98.77	18.25–53.00		
1-month survival								0.028
Alive	79	42.413	28.740	33.070	2.321–129.560	23.900–54.160		
Deceased	12	25.241	16.501	20.015	3.913–56.110	15.244–34.435		

NGAL: Neutrophil gelatinase-associated lipocalin; SD: Standard deviation; Min: Minimum; Max: Maximum.

Table 4. Relationship of NGAL levels with tomography findings

Variables	n	NGAL level					p
		Mean	SD	Median	Min–Max	25–75 quartiles	
Tomography findings							0.88
Mild	28	48.907	32.921	40.140	3390–129 560	28.335	
Moderate	31	39.004	27.046	35.510	2321–12 330	18.859	
Severe	32	33.594	22.521	28.580	3913–90 530	17.451	

NGAL: Neutrophil gelatinase-associated lipocalin; SD: Standard deviation; Min: Minimum; Max: Maximum.

There was no significant relationship between serum NGAL levels and demographic characteristics. A significant difference was found between 1-month survival and serum NGAL levels, and the median NGAL levels of patients who survived and those who did not (33 070 and 20 015, respectively; p=0.028) (Table 3).

In the analysis performed to determine the relationship between NGAL levels and tomographic findings, the median NGAL levels (40 140, 35 510, and 28 580, respectively) compared to mild, moderate, and severe tomographic involvement were not statistically significant, but median NGAL levels were found to be lower as tomographic involvement became severe (Table 4).

In patients who needed pulse steroid therapy compared to patients who did not, median serum NGAL levels (24.70 and 35.51, respectively; p=0.034) were found to be low.

A significant correlation was found between serum NGAL levels and the need for high-flow nasal oxygen therapy. The mean serum NGAL level of the 35 patients who needed high-flow nasal oxygen therapy was 33.05 mg/dL, while

Table 5. Relationship of NGAL levels with treatment and outcome findings

Variables	n	NGAL level					z	p
		Mean	SD	Median	Min–Max	25–75 quartiles		
Hydroxychloroquine							-1.906	0.057
Not used	44	34.61	24.35	29.21	2.32–105.52	17.36–46.48		
Used	47	45.33	30.35	35.29	3.39–129.56	25.46–56.11		
Corticosteroids							-0.009	0.993
Not used	29	40.13	29.57	32.09	2.32–127.28	24.24–44.92		
Used	62	40.15	27.46	30.63	3.91–129.56	18.81–54.47		
Pulse Glucocorticoids							-2.124	0.034
Not used	64	43.87	29.99	35.51	2.32–129.56	25.46–53.98		
Used	27	31.31	30.39	24.70	5.09–75.55	17.28–51.66		
Enoxaparin								
Used	91	40.14	27.98	31.90	2.32–129.56	21.17–53.00		
Dipyridamole							-0.087	0.931
Not used	86	40.09	28.04	31.41	2.32–129.56	21.62–52.93		
Used	5	41.09	30.16	35.61	5.09–73.05	12.98–71.94		
Colchicum							-0.667	0.505
Not used	66	40.56	27.35	32.73	3.39–129.56	23.07–53.12		
Used	25	39.03	30.14	30.14	2.32–105.52	17.36–59.37		
HFNO							-2.194	0.028
Not used	56	44.58	29.64	35.56	2.32–129.56	25.53–53.84		
Used	35	33.05	23.82	27.05	3.91–90.53	16.46–53.00		

No statistical comparison was made because there were no patients who did not use enoxaparin. NGAL: Neutrophil gelatinase-associated lipocalin; HFNO: High flow nasal oxygen therapy; SD: Standard deviation; Min: Minimum; Max: Maximum.

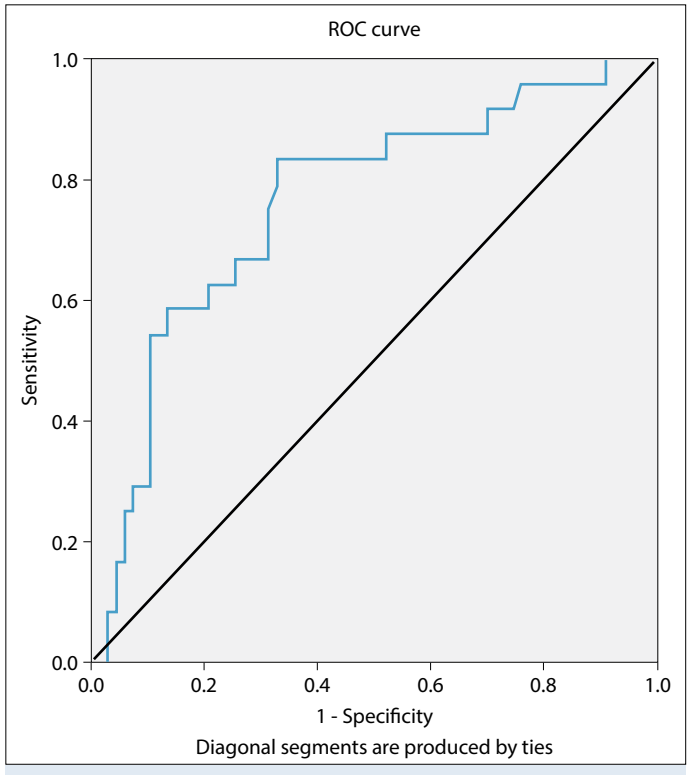

Figure 1. Receiver operating characteristic curve showing the relationship between NGAL levels and the need for intensive care.

Table 6. Relationship between NGAL levels and length of hospital stay

Variables	NGAL level	
	r	p
Symptom duration, days	-0.251	0.016
Length of stay, days	-0.316	0.02

r: Correlation coefficient (calculated between numerical variables). NGAL: Neutrophil gelatinase-associated lipocalin

the mean serum NGAL level of the 56 who did not was 44.58 mg/dL. The median serum NGAL levels were 27.05 mg/dL and 35.56 mg/dL, respectively (p=0.028) (Table 5).

Spearman's correlation coefficient was examined to determine whether there was a significant relationship between symptom duration and serum NGAL level. According to the results of the correlation analysis, a statistically significant relationship was found between serum NGAL levels and symptom duration (r=-0.251, p=0.016).

Similarly, the results of Spearman's correlation coefficient analysis for length of stay and serum NGAL level determined a statistically significant relationship between the two (r=-0.316, p=0.002) (Table 6).

Table 7. Relationship between NGAL levels and prognostic findings

Variables	Univariable OR (95% CI)	p	Multivariable OR (95% CI)	p
Age	1.0555 (1.01–1.10)	0.007*	1.06 (1.00–1.12)	0.024*
Gender		0.225		0.194
Female	1		1	
Male	0.54 (0.20–1.44)		0.43 (0.12–1.52)	
Hypertension		0.073		0.093
Absent	1		1	
Present	2.50 (0.91–6.81)		0.91 (0.22–3 .75)	
Diabetes mellitus		0.342		0.603
Absent	1		1	
Present	1.57 (0.61–4.03)		1.38 (0.40–4.67)	
Fever		0.006*		0.045*
Absent	1		1	
Present	4.08 (1.48–11.20)		3.49 (1.02–11.87)	
Loss of taste or smell		0.192		0.682
Absent	1		1	
Present	3.04 (0.57–16.26)		1.62 (0.16–16.48)	
NGAL level		<0.0001*		<0.0001*
≥30.29	1		1	
<30.29	10.22 (3.11–33.56)		10.93 (2.92–40.85)	

NGAL: Neutrophil gelatinase-associated lipocalin; *: Statistically significant values; OR: Odd ratios; CI: Confidence interval.

The median NGAL level (18.76 and 37.95, respectively; p<0.001) showed a significant difference in patients requiring intensive care treatment compared to patients admitted to the ward.

ROC analysis was performed to show the relationship between serum NGAL levels and the need for treatment in the intensive care unit (Fig. 1). The cutoff NGAL level was found to be 30.29 (Table 7). Patients with serum NGAL levels below 30.29 were found to have a 10.9-fold increased risk of entering the ICU in the multivariate analysis compared to those with a serum NGAL level above 30.29. At the same time, patients with fever were 3.49 times more likely to be admitted to intensive care than those without. It was observed that the risk of admission to intensive care increased with age.

Discussion

This study examined the relationship between the clinical course of COVID-19 and serum NGAL levels. It was determined that in patients with mild/moderate pneumonia and severe pneumonia, serum NGAL levels measured from samples taken on day 8 after symptom onset were closely related to the prognosis of the disease. In addition, serum NGAL levels had a stronger prognostic value in terms of indicating admission to the intensive care unit compared to CRP, procalcitonin, IL-6, fibrinogen, and ferritin parameters, which are frequently used in the clinic.

Coronavirus disease 2019, coronavirus 2 (SARS-CoV-2) is a highly heterogeneous disease. In addition to viral load and genotype, host characteristics also contribute to this heterogeneity.[9] Recent research has shown that a large number of mutations detected in the COVID-19 virus and viral genetic diversity can both contribute to variable infection severity and death.[10]

The effect of the innate immune system on the prognosis of COVID-19 is supported by many studies.[11–14] In a study investigating the expression of transcription factors that can cause severe inflammation, it was found that genes that are specifically upregulated in critically ill patients mainly belong to the NF-κB pathway.[15] The protein NGAL, used in the present study, is regulated through the NF-kB pathway[3,16] and was thus investigated as an important innate immune element.

In many studies that argue that iron metabolism is an important point in the pathogenesis of COVID-19, it has been reported that iron is an essential micronutrient for both humans and pathogens, and therefore iron is an important trace element in COVID-19.[17] Iron is required for viral replication and other processes such as mitochondrial function, ATP production, DNA/RNA synthesis and repair, and cell survival/ferroptosis.[18] Liu et al.[19] argued that iron chelation may be an important treatment regimen for COVID-19, as SARS-CoV-2 needs iron for its viral replication and functions.

An essential element of innate immunity, NGAL is a critical iron regulatory protein in inflammatory conditions. It even inhibits bacterial growth by preventing bacteria from obtaining siderophore iron.[20] Learned data from other viral diseases show that iron overload leads to a worse prognosis in HBV and HCV viral infections, and iron supplementation is associated with poor prognosis in HIV patients, independent of the anemic condition.[21–23] In addition, in a meta-analysis evaluating iron metabolism and anemia in COVID-19, it was stated that innate immunity may restrict the iron in plasma during infections in a way that deprives them of pathogens.[24]

It is known that NGAL is an important innate immune protein in defense against iron-using pathogens.[25,26] To investigate this efficacy, Berger et al.[5] associated gene-targeted lipocalin 2 (NGAL)-deficient mice (Lcn2 –/– mice) in vivo with increased susceptibility to bacterial infections. They demonstrated that neutrophils isolated from Lcn2 –/– mice showed significantly less bacteriostatic activity compared to WT controls, and the bacteriostatic property of WT neutrophils was eliminated by the addition of exogenous iron. This indicates that the main function of lipocalin 2 in the antibacterial innate immune response is to limit iron.

In the present study, it was observed that patients with low NGAL levels for any reason had an increased need for intensive care hospitalization for COVID-19, in which iron metabolism plays an important role.

There are many reports in the literature of markedly elevated serum NGAL levels during bacterial infections.[27–29] In the present study, the risk for patients with NGAL levels lowers than 30.29 to require intensive care was increased 10.9 times. This may seem to contradict previous studies showing an increase in NGAL levels during infection; however, in a study by Landrø et al.,[4] decreases in NGAL levels were shown in untreated HIV-infected patients, and serum NGAL levels were found to increase gradually with treatment in patients receiving HAART. These findings suggest a link between low NGAL levels, impaired neutrophil chemotaxis and killing activity, and defective neutrophil degranulation, as in HIV-infected patients.

In a study examining the similarity of diseases caused by COVID-19 and HIV viruses, a mechanism known as NETosis was emphasized. NETosis is a neutrophil death mechanism in which neutrophils release chromatin fiber networks into the extracellular space. These networks contain histones, antimicrobial peptides, or oxidizing enzymes. Li et al.[30] investigated the importance of NETosis and NGAL on infections. They determined that NGAL is a component of NETs as there were NETs that stained positive for NGAL, particularly at the infection site.

The reason for the low serum NGAL levels in the present study may be due to a similar mechanism. These low levels may have been due to an insufficient innate immune response, decreased neutrophil activation caused by infection, or consumption-related decreases because of NETosis as NGAL is intensely present in NETs. As a result, the need for intensive care seemed to be increased in patients with low NGAL.

This study did not determine the reason for the low NGAL levels, as serial measurements were not taken during the course of the disease.

ROC analysis was performed to illustrate the relationship between serum NGAL levels and the need for treatment in the intensive care unit, and the cutoff NGAL level was found to be 30.29. This parameter shows that the NGAL level obtained at the time of admission due to COVID-19 is an important predictor of prognosis and is a valuable parameter in terms of hospitalization decision.

In this study, those with an NGAL level below 30.29 had a 10.9-fold increased risk of entering intensive care compared to those with a level above 30.29, according to multivariate analysis.

As far as is known, this study is the first to investigate serum NGAL levels as prognostic indicators in COVID-19 pneumonia and is thus valuable in this respect.

The small number of patients and the cross-sectional nature of the study are its major limitations. Because it was a cross-sectional study, how serum NGAL levels progressed according to treatment and clinical follow-ups could not be determined. A further limitation is the fact that the basal serum NGAL levels of the patients were not known.

Larger-scale studies that include urinary and tracheal NGAL levels as well as serum NGAL levels will help to explain the findings.

The prospective nature of the study, the fact that serum NGAL levels were taken on the eighth day of similar symptoms, and the fact that the 1-month survival rates were included are considered strengths of this study.

As a result of the research presented herein, measuring the serum NGAL level at the time of admission for patients with COVID-19 pneumonia will be critical in terms of predicting the prognosis of the disease.

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