Research Article

Guzin Aykal*, Hatice Esen, Derya Seyman and Tuğba Çalışkan

Could IL-6 predict the clinical severity of COVID-19?

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Abstract

Objectives: An excessive inflammatory response to SARS-CoV-2 is thought to be a major cause of disease severity in COVID-19. The aim herein was to determine the prognostic value of IL-6, and demonstrate the comparison between IL-6 and related parameters in COVID-19.

Methods: Data were collected from 115 COVID-19 patients.

Results: The median age was 46.04 years in the mild group, 56.42 years in the moderate group, and 62.92 years in the severe group ($p=0.001$). There was a significant difference in the hospitalized clinic to intensive care unit ratio among the patients ($p<0.001$). The IL-6 values were significantly higher in the severe group than those in the mild ($p=0.04$) and moderate groups ($p=0.043$). The area under the receiver operating characteristic curve for IL-6, as predictor of severe clinical condition, was 0.864 (95% CI 0.765–0.963 $p=0.000$). The longitudinal analyses showed that the severe group presented with significantly increased IL-6 levels during hospitalization.

Conclusions: IL-6 seemed to be a guide in the early diagnosis of severe COVID-19 and an ideal marker for monitoring negative outcome.

Keywords: COVID-19; cytokine storm; inflammatory parameters; interleukin-6.

Introduction

A highly contagious and lethal respiratory disease, which emerged in Wuhan, China, was declared an international pandemic outburst of pneumonia named coronavirus disease 2019 (COVID-19) [1–3]. From a clinical aspect, the greater majority of COVID-19 infected patients (80%) are either completely asymptomatic or present with mild patterns. However, approximately 15% of COVID-19 infected patients experience a severe form of the disease that necessitates oxygen support, while about 5% experience a highly critical and life-threatening form of the disease, in addition to complications, which can include acute respiratory distress syndrome, respiratory failure, sepsis and septic shock, and thromboembolism, in addition to multiorgan failure, which includes acute kidney and cardiac injury [2, 4, 5].

The causes behind the development of severe or critical forms of COVID-19 infection in individuals who are infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have not yet been fully understood, and the progress of the severe form of the disease seems to be related to more than just the viral load, and may possibly be interrelated with a defective interferon response [6, 7]. The occurrence of an excessive inflammatory response to SARS-CoV-2 is believed have a significant effect on disease severity and death in patients who are infected with COVID-19, and has been shown to have an association with high circulating cytokines levels, severe lymphopenia, and a substantial degree of mononuclear cell infiltration in the lungs, spleen, heart, kidney, and lymph nodes, as observed through postmortem analyses [6, 8].

Macrophage activation syndrome, also known as acquired (secondary) hemophagocytic lymphohistiocytosis, which is characterized by cytokine storm due to a hyper-inflammatory response, has been reported in some patients with a severe clinical state of COVID-19 [9–12].

Cytokines have a significant role in the regulation of immunological and inflammatory responses, in addition to the pathogenesis of a cytokine storm [13, 14]. Interleukin-6 (IL-6), which is a cytokine, is a key mediator of the immune and inflammatory response that is triggered by injury or infection. IL-6 levels have been determined to be a very important predictor of severe infection in COVID-19 [15–17].

***Corresponding author: Guzin Aykal**, Tıbbi Biyokimya Kliniği Antalya Eğitim ve Araştırma Hastanesi, Varlık Mahallesi, Kazım Karabekir Cd., 07100 Muratpaşa, Antalya, 07100, Turkey,
E-mail: guzinaykal@yahoo.com.

<https://orcid.org/0000-0002-2413-2695>

Hatice Esen, ARGE Birimi, Antalya Eğitim ve Araştırma Hastanesi, Antalya, Turkey

Derya Seyman, Enfeksiyon hastalıkları ve klinik mikrobiyoloji kliniği, Antalya Eğitim ve Araştırma Hastanesi, Antalya, Turkey

Tuğba Çalışkan, Aile Hekimliği, Antalya Eğitim ve Araştırma Hastanesi, Antalya, Turkey

IL-6, which is a pleiotropic cytokine, has the ability to affect a number of immune and physiological processes, including the production of acute phase protein and inflammation, and the triggering of antigen-specific immune responses, apoptosis, hematopoiesis, differentiation, as well as cellular metabolism [18, 19].

IL-6 also contributes to host defense against tissue damage and infection. However, there is a significantly increased levels of IL-6 released when the body fights against SARS-CoV-2, and this very high level triggers an acute and severe hyperactive immune response that is called a cytokine storm [18, 20].

The aim of the study presented herein was to conduct an investigation of the role that IL-6 levels play in COVID-19 diagnosis and treatment, as well as to identify patients with confirmed COVID-19 disease who could be at the high risk of severe inflammatory response and demonstrate the comparison between IL-6 and other laboratory parameters of inflammation in patients who are infected with COVID-19.

Materials and methods

Study design

In this retrospective, single-center study, data were collected from 115 patients who were admitted to the Antalya Education and Research Hospital between 11 March 2020 and 4 April 2020.

The study population herein comprised 60 patients who were confirmed to be positive for COVID-19 infection as the result of SARS-CoV-2 nucleic acid real-time polymerase chain reaction (RT-PCR) analysis using specimens that had been derived from nasal and oropharyngeal swabs. Additionally, 55 patients were confirmed to be positive for COVID-19 infection as the result of thorax tomography.

The diagnosis, as well as the treatment, of the COVID-19 patients were performed according to the COVID-19 (SARS-CoV-2 infection) Patient Treatment Guidelines issued by the Turkish Ministry of Health, as well as the interim guidance of the World Health Organization (WHO).

Based on the guidelines, the patients herein were categorized as given below:

- (1) Mild cases:
 - a. Symptoms such as fever, myalgia or arthralgia, cough, or sore throat, without respiratory distress (resting respiratory rates <24 breaths/min, and oxygen saturation >95%).
 - b. Chest X-ray and/or chest computed tomography (CT) scan are normal.
- (2) Moderate cases:
 - a. Symptoms such as fever, myalgia or arthralgia, cough, or sore throat, with resting a respiratory rate that was <24 breaths/min and oxygen saturation >93%.
 - b. Patients with signs of mild to moderate pneumonia on chest X-ray or CT scan (presence of radiological findings in less than 50% of the lung parenchyma; patchy shadowing, focal ground glass opacity, and interstitial abnormalities).

(3) Severe cases:

- a. Symptoms such as fever, myalgia or arthralgia, cough, or sore throat, with a resting a respiratory rate that was ≥ 30 breaths/min as well as oxygen saturation that was $\leq 90\%$.
- b. Patients with bilateral diffuse pneumonia findings on chest X-ray or CT scan (presence of radiological findings in more than 50% of the lung parenchyma; patchy shadowing, focal ground glass opacity, and interstitial abnormalities).

Patient data, comprising their demographic characteristics, any existing underlying comorbidities, symptoms exhibited, as well as laboratory and physical test results, were taken from the hospital electronic medical records and recorded.

The laboratory tests conducted comprised C-reactive protein (CRP), baseline IL-6, neutrophils (NEU), white blood cell (WBC), platelet (PLT), and lymphocyte (LYM) counts, in addition to the erythrocyte sedimentation rate (ESR), neutrophil lymphocyte ratio (N/L), and D-dimer, lactate dehydrogenase (LDH), high-sensitivity troponin T (hs-cTnT), ferritin, and procalcitonin (PCT) levels on admission to the hospital. IL-6 levels during the period of hospitalization in moderate and severe cases were included in the study.

Measurement of the body temperatures of the patients was performed using an infrared thermometer, and the diagnosis of fever was determined as a temperature that was above 37 °C.

This study was granted approval by the Institutional Research Ethics Committee (Process No. 2020-7/17).

Laboratory diagnosis

Specimens derived from both pharyngeal and nasal swabs were collected from each patient and placed individually into collection tubes that contained virus preservation solution (Bio-Speedy NAT transfer tube, specimen collection flocked swap. Bioeksan R&D Technologies Ltd., İstanbul, Turkey). Nucleic acid extraction were obtained with Bio-Speedy-nucleic acid isolation kits according to the to the manufacturer's instructions. Nucleic acid isolates were studied with real-time RT-PCR technique via using Qiagen Rotorgene real time PCR cyclers (QIAGEN, Hil-den, Germany). Bio-Speedy SARS CoV-2 RT-qPCR kit was used targeting the SARS-CoV-2 specific N and Orf1ab gene region. Cycle threshold (CT) value less than 38 is interpreted as positive for SARS-CoV-2 RNA.

Blood samples obtained in the morning during the first 24 h of hospitalization of all COVID-19 patients were included in the study. The IL-6 test results performed during the hospitalization period were evaluated together with the baseline test results. From each patient, 2.0 mL of total venous blood was collected from the antecubital vein into evacuated tubes (Greiner Bio-One, Austria) that contained 0.109 M, 3.2% trisodium citrate to determine the D-dimer levels; 2.0 mL of total venous blood was collected from the antecubital vein into evacuated tubes (Isotherm Co. Ltd., Turkey) that contained K3EDTA to determine the WBC, NEU, LYM, and PLT counts, N/L, and ESR; and 5.0 mL venous blood was collected into tubes that contained a spray-dried clot activator and gel separator (Isotherm Co. Ltd. Turkey) to measure the IL-6, CRP, hs-cTnT, LDH, ferritin, and PCT levels.

Separation of the citrated plasma and serum samples from the cells was conducted via centrifugation for 10 min at $1653 \times g$ Relative Centrifugal Force (RCF), and all of the parameters were measured recurrently.

IL-6 was measured using commercially available kits on a Cobas e411 Immunoassay Analyzer (Roche Diagnostics, Germany). Analytical

performance data of IL-6 measured by the electrochemiluminescence immunoassay method were as follows: Measuring interval was 1.5–5000 pg/mL (defined by the lower detection limit and the maximum of the master curve). The reference range was up to 7 pg/mL IL-6 (95th percentile). Precision: The repeatability values for human serums at different levels were as follows; 17.3 pg/mL CV% 6.0, 117 pg/mL CV% 2.5 and 891 pg/mL CV% 2.6 Analytical specificity: IL-6 assay does not show any significant cross-reactivity with the IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-8 IFN- γ , TNF- α , tested with IL-6 concentrations of approximately 3 pg/mL and 4000 pg/mL (maximum tested concentration)

Measurement of the CRP and LDH levels was conducted using kits that were commercially available with a fully-automated AU 5800 analyzer (Beckman Coulter Inc., USA).

The WBC, NEU, LYM, and PLT counts, and N/L were determined using kits that were commercially available with an XN 1000 auto-analyzer (Sysmex Corp. Japan).

The ESR was measured using an undiluted blood sample with ethylenediaminetetraacetic acid evacuated tubes with a fully-automatic Vision-C analyzer (YHLO Biotech Co., China).

hs-cTnT, levels were conducted kits that were commercially available with a fully-automated Cobas e411 analyzer (Roche Diagnostics, Germany)

The D-dimer levels were measured using commercially available kits with a fully-automated ACL TOP 700 analyzer (Instrumentation Laboratory Co., USA).

Ferritin was measured using commercially available kits with an a fully-automated DXI 800 analyzer (Beckman Coulter Inc., USA).

The PCT levels were determined using commercially available kits with a fully-automated Cobas 8000 analyzer (Roche Diagnostics, Germany)

Statistical analysis

Statistical analyses of the data obtained herein were conducted using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Analytical methods were used to determine whether or not the variables were normally distributed. Descriptive analyses were presented using medians and interquartile (IQR) for the non-normally distributed and ordinal variables. Since the IL-6 measurement was not normally distributed, the Mann-Whitney U and Kruskal-Wallis tests were used to compare IL-6 and laboratory results between the groups. Multivariate logistic regression analysis was used to determine independent risk factors associated with severe COVID-19. The variables with $p < 0.2$ in the univariate analyses were further tested in the multivariate model. Odds ratio (OR) with corresponding 95% confidence intervals (95% CIs) was reported.

Results

Demographic and clinical characteristics

The study population herein comprised total of 115 patients, including 46 females (40%) and 69 (60%) males, who had received a diagnosis of COVID-19 infection. Of these 115 patients, 24 were classified as mild, 52 as moderate, and 39 as severe. There were no significant differences in the male

to female ratio between the three groups ($p = 0.566$). The relationship between mild, moderate and severe clinical conditions and age group cases was analyzed by dividing them into three age groups (20–39, 40–59 and up to 60 years old). It has been determined that the clinical condition was more severe with increasing age ($p = 0.008$).

It was determined that there was a significant difference in the hospitalized clinic to intensive care unit ratio among mild, moderate, and severe groups ($p < 0.001$).

More than half of the patients (66.9%) had, at minimum, one comorbidity. It was determined that hypertension (31.3%) was the most common comorbidity, after which was diabetes (29.5%).

Among the comorbidities, the rate of asthma was determined to be significantly different in all of the clinical groups ($p = 0.010$).

Cough (58.3%), shortness of breath (43.5%), and fever (79.72%) were the most common symptoms, while fatigue (16.5%), gastrointestinal symptoms (diarrhea, vomiting, etc., 10.4%) and sore throat (7.8%) were relatively less common. Significant differences were determined between the three study groups with regards to shortness of breath and myalgia ($p = 0.042$ and $p = 0.003$).

Table 1 presents the demographic and clinical characteristics of the patients in all of the groups.

Clinical laboratory data

60 patients included in the study were confirmed to be positive for COVID-19 infection as the result of SARS-CoV-2 nucleic acid real-time polymerase chain reaction (RT-PCR) analysis. 55 patients were confirmed to be negative for COVID-19 as the result of SARS-CoV-2 RT-PCR. However, thoracic tomography findings of these patients were consistent with COVID-19.

Table 2 presents the comparisons obtained by performing Kruskal Wallis for different parameters among the different clinical situations of the patients on admission. In this study, which covered the first months of the pandemic, there was no standardization regarding the test request yet. Test requests were made according to the symptoms and clinical findings of the patients, and the number of patients in each parameter was different. Age ($p < 0.001$) and IL-6 test results ($p = 0.011$) differed significantly according to the clinical classification of the disease. The mean age of the patients was 56.4 years. The median age for the mild group was 46.04 years (range 21–72 years), while it was 56.42 years for the moderate group (range 21–85 years), and 62.92 years (range 24–93) for the severe group ($p < 0.001$). Post-hoc test was performed among age groups (mild < moderate, severe).

Table 1: Demographic data and initial clinical characteristics in the study cohort.

		Mild		Moderate		Severe		Test	p
		n	%	n	%	n	%		
Age	20–39	11	45.8%	11	21.2%	3	7.7%	13.85	0.008
	40–59	7	29.2%	15	28.8%	16	41%		
	60 ↑	6	25%	26	50%	20	51.3%		
Gender	Female	10	42%	23	44%	13	33%	1.138	0.566
	Male	14	58%	29	56%	26	67%		
Clinical outcomes	Hospitalization	24	100%	37	71%	12	31%	33.13	<0.001
	Intensive care unit	0	0%	15	28.8%	27	69.2%		
Chronic medical illness									
Hypertension	Yes	6	25%	17	33%	13	33%	0.565	0.754
	No	18	75%	35	67%	26	67%		
Diabetes	Yes	6	25%	17	33%	11	28%	0.519	0.771
	No	18	75%	35	67%	28	72%		
Cancer	Yes	2	8%	4	8%	3	8%	0.199	0.999
	No	22	92%	48	92%	36	92%		
Hepatitis c	Yes	0	0%	1	2%	1	3%	0.761	0.999
	No	24	100%	51	98%	38	97%		
Chronic obstructive pulmonary disease (COPD)	Yes	3	13%	3	6%	2	5%	1.515	0.560
	No	21	88%	49	94%	37	95%		
Asthma	Yes	6	25%	3	5.8%	1	2.6%	8.274	0.010
	No	18	75%	49	94%	38	97%		
Coronary heart disease	Yes	1	4%	8	15%	9	23%	4.029	0.133
	No	23	96%	44	85%	30	77%		
Rheumatic disease	Yes	0	0%	1	2%	0	0%	1.371	0.999
	No	24	100%	51	98%	39	100%		
Chronic renal disease	Yes	0	0%	0	0%	0	0%	0.644	0.846
	No	23	96%	49	94%	38	97%		
Epilepsy + Alzheimer + cerebrovascular diseases	Yes	1	4%	2	4%	4	10%	1.635	0.533
	No	23	96%	50	96%	35	90%		
Symptoms									
Fever	Yes	7	29%	17	33%	17	44%	1.71	0.425
	No	17	71%	35	67%	22	56%		
Cough	Yes	14	58%	29	56%	24	62%	0.305	0.859
	No	10	42%	23	44%	15	39%		
Shortness of breath	Yes	7	29%	20	39%	23	59%	6.344	0.042
	No	17	71%	32	62%	16	41%		
Weight	Yes	3	13%	4	8%	2	5%	1.238	0.541
	No	21	88%	48	92%	37	95%		
Sputum production	Yes	0	0%	4	8%	3	8%	1.748	0.474
	No	24	100%	48	92%	36	92%		
Weakness	Yes	2	8%	10	19%	7	18%	1.501	0.472
	No	22	92%	42	81%	32	82%		
Headache	Yes	2	8%	3	6%	2	5%	0.543	0.779
	No	22	92%	49	94%	37	95%		
Diarrhea + abdominal pain + nausea + vomiting	Yes	1	4%	9	17%	2	5%	4.109	0.127
	No	23	96%	43	83%	37	95%		
Sore throat	Yes	1	4%	5	10%	3	8%	0.56	0.903
	No	23	96%	47	90%	36	92%		
Myalgia	Yes	0	0%	9	17.3%	0	0%	10.646	0.003
	No	24	100%	43	83%	39	100%		
Consciousness	Yes	0	0%	2	4%	2	5%	0.966	0.820
	No	24	100%	50	96%	37	95%		

The bold values in tables are statistically significant.

Table 2: The comparisons obtained by performing Kruskal–Wallis for different parameters among different clinical situations of the patients on admission.

		n	Median (Q1–Q3)	P
Age	Mild	24	42 (31–59)	0.001
	Moderate	52	59 (41–71)	
	Severe	39	61 (54–79)	
IL-6, pg/mL	Mild	24	18 (6–66)	0.011
	Moderate	52	45 (6–97)	
	Severe	39	71 (31–143)	
PCT, ng/mL	Mild	11	0.05 (0.03–0.11)	0.08
	Moderate	28	0.14 (0.08–0.42)	
	Severe	15	0.13 (0.07–0.84)	
D-dimer mg/L	Mild	14	317 (122–617)	0.742
	Moderate	25	292 (187–1297)	
	Severe	17	248 (145–508)	
Ferritin, mg/L	Mild	14	97 (35–225)	0.07
	Moderate	28	163 (79–561)	
	Severe	18	391 (148–727)	
CRP, mg/L	Mild	13	27 (22–69)	0.359
	Moderate	27	71 (19–233)	
	Severe	17	91 (43–129)	
WBC $\times 10^9/L$	Mild	14	6.7 (6.2–12.2)	0.771
	Moderate	27	7.2 (5.2–11)	
	Severe	23	8.7 (4.8–15)	
LDH, U/L	Mild	11	212 (185–280)	0.531
	Moderate	22	240 (185–435)	
	Severe	12	257 (202–421)	
hs-cTnT, ng/L	Mild	11	10.2 (5.3–56.8)	0.994
	Moderate	22	12.2 (4.7–51.4)	
	Severe	13	10.2 (5.3–33.7)	
LYM $\times 10^9/L$	Mild	14	1.4 (0.8–1.8)	0.884
	Moderate	26	1.1 (0.9–1.6)	
	Severe	23	1.2 (0.8–1.8)	
NEU $\times 10^9/L$	Mild	14	5.2 (4.2–9.1)	0.476
	Moderate	27	4.6 (3.1–9.3)	
	Severe	23	8.1 (3.5–10.9)	
N/L	Mild	13	5.5 (3.1–7.9)	0.672
	Moderate	26	3.9 (2.4–9.9)	
	Severe	24	6.1 (2.4–10.2)	
PLT $\times 10^9/L$	Mild	14	270 (214–334)	0.417
	Moderate	27	226 (195–272)	
	Severe	23	235 (168–295)	

The bold values in tables are statistically significant.

A significant difference was determined between the mild and moderate ($p=0.035$) as well as the mild and severe ($p<0.001$) groups; however, no such difference was seen between the moderate and severe ($p=0.162$) groups. Post-hoc test was also performed on IL-6 results for all three groups (mild<severe). It was determined that a statistically significant difference existed between the mild and severe ($p=0.013$) groups; however, no such difference was seen between the mild and moderate ($p=0.738$) or the moderate and severe ($p=0.093$) groups.

Baseline IL-6 levels obtained by comparing the Mann-Whitney U test of the paired groups are shown in Figure 1. The IL-6 levels were significantly higher in the patients in the severe group than in those in the mild group ($p=0.04$). Furthermore, the IL-6 levels were increased significantly on admission in the patients in the severe group when compared with those in the moderate group ($p=0.043$, Figure 1). However, there was no difference in the baseline IL-6 levels between the patients in the mild group and those in the moderate group ($p=0.223$, Figure 1).

Table 3 presents the result of multivariate logistic regression analysis performed to identify the independent factors associated with severe COVID-19. Increasing age (OR: 1.031; 95% CI: 1.004–1.060; $p=0.026$) and increasing IL-6 (OR: 2.258; 95% CI: 1.222–4.175; $p=0.009$) were identified as significant risk factors for severe COVID-19.

Analysis by receiver operating characteristic

In order to conduct an evaluation of the prognostic value that IL-6 exhibits in patients diagnosed with COVID-19, a receiver operating characteristic (ROC) curve was drawn for the patients in the mild and severe groups. The area under the ROC curve (AUC) of IL-6, which was used for the prediction of the COVID-19 severity, was 0.864 (95% CI 0.765–0.963 $p<0.001$), and was found to more accurately predict if COVID-19 was complicated with severe pneumonia.

Figure 1: The comparisons obtained by performing Mann-Whitney U test for IL-6 results of the paired groups.

Table 3: The result of multivariate logistic regression analysis performed to identify the independent factors associated with severe COVID-19.

Variables	Or (95% CI)	p
Age	1.031 (1.004–1.06)	0.026
Asthma	0.119 (0.012–1.148)	0.066
Coronary heart disease	0.904 (0.263–3.112)	0.873
Shortness of breath	2.053 (0.804–5.242)	0.133
Diarrhea + abdominal pain + nausea + vomiting	0.379 (0.067–2.152)	0.273
IL-6	2.258 (1.222–4.175)	0.009

Moreover, calculation of the specificity, sensitivity, and predictive positive and negative IL-6 values was performed to determine the optimal threshold value, which was found to be 28 pg/mL (Figure 2).

The available longitudinal IL-6 measurement data of the patients in the subgroups were analyzed to conduct an investigation of their serial changes with regards to the severity of their COVID-19 infection. As a result, it was

determined that only measurement of the baseline IL-6 levels was performed in patients who had mild COVID-19 infection. The levels of IL-6 were taken at five time intervals and presented. Day 0 was the first time interval, which comprised the baseline concentrations of the patients, taken within the first 24 h after admission. Measurements were conducted every other day for patients who were diagnosed with moderate and severe COVID-19 infection. When a comparison was conducted between the moderate and severe groups, it was determined that the patients who were in the severe group exhibited significantly increased serum concentrations of IL-6 during the course of hospitalization (Figure 3). The data presented in Figure 3 were determined by using the mean values of longitudinal IL-6 results. The IL-6 levels of five patients who did not survive COVID-19 were examined at most three times. The baseline IL-6 levels of these five expired patients were 59.48, 58.98, 115.2, 1408, and 20.95 pg/mL, respectively. All suspected or confirmed cases with COVID-19 were received hydroxychloroquine (or plus azithromycin/oseltamivir) on admission, according to the national protocol of Ministry of Health. As from 01 April 2020, favipiravir was introduced, favipiravir was started to be used for severe cases. Tocilizumab was not used during study period.

Figure 2: Receiver operating characteristic (ROC) curve showing the predictive power of IL-6 for predicting severe COVID-19.

Figure 3: Longitudinal changes of serum IL-6 levels in moderate and severe COVID-19 patients.

Discussion

The first studies from China pointed to gender inequality when an examination of the detected cases and case mortality rates was conducted for COVID-19 [3, 21]. The WHO reported in the following period that through a preliminary analysis of the available data, it was determined that there was a somewhat even distribution with regards to COVID-19 infections between females and the males (47 vs. 51%, respectively) in the study population, and some variations were observed across the different age groups [22]. Novel evidence on the progress of COVID-19 and its severity has shown that the hospitalization rate is 50% higher for males than it is for females [21, 23]. The current study showed that the number of men (60%) infected with COVID-19 was 1.5-fold higher than the number of women (40%). This may have been caused by reasons such as gender-specific steroids and the activity of X-linked genes, which modulate the innate and adaptive immune responses [24]. The gender distribution data considered in our study are compatible with the literature [21, 23]. However, the low number of cases in these data belonging to the beginning period of the pandemic is the weakness of our study.

Old age is the most important risk factor in terms of the severe clinical situation and mortality in COVID-19 [21, 25, 26]. Some studies have reported that among the patients who had been hospitalized as a result of COVID-19, 74–86% were over 50 years old [4]. Patient mortality rates are the highest in patients who were over 80 years old, while they are the lowest in patients who were under 40 years old [4, 21]. In the current study, the mean age of the hospitalized patients was determined as 56.4 years, and it was seen that as the

disease severity in the patients became more severe, the mean age increased. This clinical status observed in the elderly could have been the result of delayed and/or dysfunction of their immune response [18, 27].

As in the literature [21, 24, 27], most of the patients in the current study had at least one comorbid disease. This study showed that the patients generally have the initial symptoms of fever, consisting of cough, shortness of breath on admission. It was observed that shortness of breath and myalgia were both more pronounced in patients who exhibited severe COVID-19 infection than in the patients in the other groups.

This retrospective cohort study identified IL-6 levels and related parameters on admission and IL-6 levels during hospitalization so as to be able to predict the severity of the COVID-19 infection in adults in Antalya, who were hospitalized with COVID-19. The elderly patients and those with high IL-6 levels had a more severe clinical picture (Tables 2 and 3).

A cytokine storm, which is also known as cytokine release syndrome (CRS), is an uncontrolled hyper-inflammatory response that results when a localized inflammatory response is spread systemically in reaction to a bacterial or viral infection [27–29]. In patients who have been infected with SARS-CoV-2, a cytokine storm significantly affects the pathogenesis of many very severe COVID-19 manifestations, which include acute respiratory distress syndrome, thromboembolic diseases, like acute ischemic strokes that result from the occlusion of large vessels and myocardial infarction, acute kidney injury, encephalitis, and vasculitis, comprising a Kawasaki-like syndrome in seen in children and renal vasculitis seen in adults [28]. IL-6, which is a key pro-inflammatory cytokine that is involved in the infection onset and progression of a disease, is especially significant during a cytokine storm in COVID-19 [25, 29, 30].

IL-6, which is a glycopeptide that is secreted by 25-kDa, consists of 184 amino acids [30–32]. IL-6 is a pleiotropic cytokine that is part of the proinflammatory cytokine family. It has an influence on a number of immune and physiological processes, including the production of acute phase proteins, such as CRP and ferritin, antigen-specific immune responses, inflammation, apoptosis, differentiation, hematopoiesis, and cellular metabolism [29, 31, 32]. IL-6 is mainly synthesized and secreted by monocytes and macrophages; however, T and B cells, hepatocytes, fibroblasts, endothelial cells, mesangial cells, adipocytes and keratinocytes, in addition to a number of tumor cells, can all produce IL-6 [29, 31, 32].

Several studies have reported elevated sera IL-6 levels in patients diagnosed with COVID-19 infection, and determined that the sera IL-6 circulating levels were positively correlated with severity and mortality in COVID-19 [25, 29]. IL-6 also contributes to host defense against tissue damage and infection. However, the occurrence of exaggerated and excessive IL-6 synthesis in response to SARS-CoV-2 infection results in the acute and severe systemic inflammatory response, known as a cytokine storm [18].

The results of this study indicated that the values of IL-6 were determined to be significantly higher in the severe group than in the mild ($p=0.04$) and moderate groups ($p=0.043$, Figure 1). The AUC for IL-6, as a predictor of the severe clinical condition, was 0.864 (95% CI 0.765–0.963, $p<0.001$; Figure 2). It was determined that the occurrence of even moderately elevated levels of IL-6, such as above 28.44 pg/mL, was sufficient to be able to identify patients who had COVID-19 infection complicated with severe pneumonia.

Various studies have suggested that COVID-19 patients should be monitored by measuring cytokines and inflammatory markers at baseline and during hospitalization [33, 34]. Moreover, it was suggested that it might be important to perform a serial measurement of the circulating IL-6 levels in patients towards the identification of the progression of the disease. Furthermore, when these levels are evaluated immediately after the confirmation of a diagnosis of COVID-19 infection, they may aid in the prediction of forthcoming respiratory failure or asymptomatic disease in patients infected with SARS-CoV-2 [30].

The longitudinal analyses showed that when compared to the patients in both moderate and severe groups, those in the severe group presented with significantly increased serum concentrations of IL-6 during the course of hospitalization. These elevated levels of cytokines may have also been responsible for the fatal complications that are commonly observed with COVID-19 infection [25]. Consequently, the baseline IL-6 levels of the patients who expired in the current study were at least 3-fold higher than the reference value.

It was observed that elevated levels of IL-6 have also been reported in a number of other studies of COVID-19 [35], which may be useful as a predictive biomarker for COVID-19 disease severity [25, 36].

In conclusion, IL-6 is a key marker of inflammation and can be used to help identify patients with confirmed COVID-19 disease who could be at high risk of severe COVID-19. Based on the results herein, IL-6 levels on hospital admission seemed to be a good predictor for the progression to severe disease and it appeared to be an ideal marker for monitoring negative outcomes. Therefore, it is herein recommend that IL-6 and related inflammation parameters should be measured at baseline and monitored during hospitalization. As a result, mortality and morbidity rates can be reduced by applying the necessary treatment options to the patient immediately.

This study had some limitations. First, only 115 hospitalized patients were included. Since it was a retrospective study, the study conducted herein did not comprise a population of control subjects. The strengths of the study included measuring the IL-6 levels in all clinical situations, comparing the IL-6 levels with all of the other inflammatory parameters, demonstrating the diagnostic value by ROC analysis, and including measurements during hospitalization. To the best of our knowledge, this study was the first conducted on the IL-6 levels in COVID-19 patients for this geographical region. It is our belief that this will contribute to the understanding of regional and racial differences in the COVID-19 pandemic.

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References

1. Madabhavi I, Sarkar M, Kadakol N. CoviD-19: a review. *Monaldi Arch Chest Dis* 2020;90:248–58.
2. Danwang C, Endomba FT, Nkeck JR, Wouna DLA, Robert A, Noubiap JJ. A meta-analysis of potential biomarkers associated with severity of coronavirus disease 2019 (COVID-19). *Biomark Res* 2020;8:1–13.
3. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;382:1708–20.
4. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *J Am Med Assoc* 2020;324:782–93.

5. WHO. Clinical management of COVID-19 interim guidance. (2020). WHO/2019-nCoV/clinical/2020.5. Available from: <https://apps.who.int/iris/bitstream/handle/10665/332196/WHO-2019-nCoV-clinical-2020.5-eng.pdf> [Accessed Oct 2020].
6. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol* 2020;20:355–62.
7. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson J. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395:1033–4.
8. Wang C, Xie J, Zhaob L, Feia X, Zhanga H, Tanet Y, et al. Alveolar macrophage dysfunction and cytokine storm in the pathogenesis of two severe COVID-19 patients. *EBioMedicine* 2020;57:102833.
9. Bakanlıđı TCS. COVID-19 (SARS-CoV-2 enfeksiyonu- bilim kurulu çalışması) antitoksin-antiinflamatuvar tedaviler. *Koagulopati Yönetimi* 2020;9:1–21.
10. Ravelli A, Davi S, Minoia F, Martini A, Cron R. Macrophage activation syndrome. *Hematol Oncol Clin N Am* 2015;29:927–41.
11. Crayne CB, Albeituni S, Nichols KE, Cron RQ. The immunology of macrophage activation syndrome. *Front Immunol* 2019;10:1–11.
12. McGonagle D, Sharif K, Regan AO, Bridgewood C. Autoimmunity reviews the role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev* 2020;19:1–7.
13. Coomes EA. Interleukin-6 in Covid-19 : a systematic review and meta- analysis. *Rev Med Virol* 2020;30:1–9.
14. Del Valle DM, Kim-Schulze S, Huang H-H, Beckmann ND, Nirenberg S, Wang B. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med* 2019;26:1636–43.
15. Aziz M, Rawish F, Ragheb A. Elevated interleukin-6 and severe COVID-19: a meta-analysis. *J Med Virol* 2020;92:2283–85.
16. Grifoni E, Valoriani A, Ceï F, Lamanna R, Gelli AMG, Ciambotti B, et al. Interleukin-6 as prognosticator in patients with COVID-19. *J Infect* 2020;81:452–82.
17. Herold T, Jurinovic V, Arnreich C, Lipworth BJ. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol* 2020;146:128–36.e4.
18. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg Microbes Infect* 2020;9:1123–30.
19. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science* 2020;80:1–5.
20. Chen G, Zhao J, Ning Q, Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020;130:2620–9.
21. Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. The impact of sex and gender on immunotherapy outcomes in Europe. *Biol Sex Differ* 2020;11:1–13.
22. WHO/2019-nCoV/Advocacy_brief/Gender/2020.1. Available from: <https://apps.who.int/iris/handle/10665/332080> [Accessed October 2020].
23. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. *Clin Infect Dis* 2021;XX:1–6.
24. Ambrosino I, Barbagelata E, Ortona E, Ruggieri A, Massiah G, Giannico OV, et al. Gender differences in patients with COVID-19: a narrative review. *Monaldi Arch Chest Dis* 2020;90:318–24.
25. Liu B, Li M, Zhou Z, Guan X, Xiang Y. Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *J Autoimmun* 2020;111:102452.
26. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020;146:110–8.
27. Liu F, Li L, Xu M, Wu J, Luo D, Zhu Y, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *J Clin Virol* 2020;127:104370.
28. Bhaskar S, Sinha A, Banach M, Mittoo S, Weissert R, Kass JS, et al. Cytokine storm in COVID-19—immunopathological mechanisms, clinical considerations, and therapeutic approaches: the REPROGRAM consortium position paper. *Front Immunol* 2020;11:1–16.
29. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev* 2020;53:25–32.
30. Gubernatorova EO, Gorshkova EA, Polinova AI, Drutskaya MS. IL-6: relevance for immunopathology of SARS-CoV-2. *Cytokine Growth Factor Rev* 2020;53:13–24.
31. Uciechowski P, Dempke WCM. Interleukin-6: a masterplayer in the cytokine network. *Oncology* 2020;98:131–7.
32. Kang S, Tanaka T, Narazaki M, Kishimoto T. Targeting interleukin-6 signaling in clinic. *Immunity* 2019;50:1007–23.
33. Yuan J, Zou R, Zeng L, Kou S, Lan J, Li X, et al. The correlation between viral clearance and biochemical outcomes of 94 COVID-19 infected discharged patients. *Inflamm Res* 2020;69:599–606.
34. Zeng Z, Yu H, Chen H, Qi W, Chen L, Chen G, et al. Longitudinal changes of inflammatory parameters and their correlation with disease severity and outcomes in patients with COVID-19 from Wuhan, China. *Crit Care* 2020;24:1–12.
35. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497–506.
36. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol* 2020;92:791–6.