Uncorrected Proof

Jundishapur J Microbiol. 2021 October; 14(10):e119060. doi: 10.5812/jjm.119060.

Published online 2021 November 18. **Research Article**

High Levels of Circulating IL-6 and IL-8 Signature can Predict COVID-19 Severity

Didem Ozgür [1,*], Murat Karamese [1], Alpay Medetalibeyoglu [2], Onur Alkan [2], Naci Senkal [2] and Emin Ediz Tutuncu [3]

[1]Department of Medical Microbiology, Faculty of Medicine, Kafkas University Kars, Kars, Turkey
[2]Department of Internal Medicine, Faculty of Medicine, Istanbul University, Istanbul, Turkey
[3]Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Kafkas University, Kars, Turkey

[*]*Corresponding author*: Department of Medical Microbiology, Faculty of Medicine, Kafkas University, 36100, Kars, Turkey. Tel: +90-5066964432, Email: dido-ozgur@hotmail.com

Received 2021 September 15; **Revised** 2021 November 06; **Accepted** 2021 November 06.

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection may trigger a cytokine storm, which is characterized by uncontrolled overproduction of proinflammatory cytokines.
Objectives: We aimed to investigate the association between circulating levels of inflammatory cytokines and severity of coronavirus disease 2019 (COVID-19).
Methods: This cross-sectional study included 46 severe and 32 mildly symptomatic COVID-19 patients. The serum levels of cytokines and chemokines were determined using the Bio-Plex Pro™ Human Cytokine Screening Panel.
Results: Out of a total of 78 patients with confirmed COVID-19, 54 (69.2%) were males, and 24 (30.8%) were females. The mean age was 43.1 ± 13.3 and 58.2 ± 15 in mild and severe patients, respectively. Severe patients were characterized by significant laboratory abnormalities, such as increased WBC ($P = 0.002$) and neutrophil counts ($P = 0.001$), higher levels of ALT ($P = 0.03$), AST ($P = 0.002$), LDH ($P < 0.001$), urea ($P = 0.013$), ferritin ($P < 0.001$), D-dimer ($P = 0.042$), CRP ($P < 0.001$), and decreased lymphocyte ($P < 0.001$) and platelet ($P = 0.045$) counts. The levels of IL-6, IL-8, IL-13, TNF-α, IFN-γ, MIP-1β, and MCP-1 increased in the severe group compared to the mild group. However, significant differences were observed only for IL-6 ($P < 0.001$) and IL-8 ($P < 0.001$) levels.
Conclusions: Serum IL-6 and IL-8 levels can be used as potential prognostic biomarkers of disease severity in COVID-19 patients.

Keywords: SARS-CoV-2, COVID-19, Cytokine Storm, Proinflammatory Cytokines, Interleukin-6, Interleukin-8

1. Background

Coronaviruses (CoVs) are enveloped with a positive single-stranded RNA, β-coronavirus belonging to the family *Coronaviridae* (1, 2). The first human coronaviruses (HCoVs) were detected in 1966; since then, they have been mainly associated with common cold or mild upper respiratory tract infections (2). In the past two decades, the severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak in 2012 caused local outbreaks and global pandemics with mortality rates of 10 and 40%, respectively (3). In December 2019, SARS-CoV-2, the third most pathogenic novel HCoV, was emerged for the first time in Wuhan, China (1, 4, 5). Coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 rapidly spread across China and many other countries, and it was declared to be a global pandemic by the World Health Organization (WHO) (6).

The clinical manifestations of COVID-19 are characterized by a variable disease phenotype, of which most patients experience only fever, cough, myalgia, fatigue, or dyspnea. About 15% of patients progress to severe disease, and acute respiratory distress syndrome (ARDS) or multiple organ failure may occur (7-9). SARS-CoV-2 infection may trigger a cytokine storm, which is characterized by the excessive production of several proinflammatory cytokines/chemokines and high-level activation of CD4+/CD8+ T cells (10). Several studies suggested that the cytokine storm directly correlated with ARDS, multiple organ failure, disease severity, and death in patients with COVID-19 (1, 9). The unfavorable prognosis of severe COVID-19 is associated with increases in several inflammatory cytokines and chemokines (9).

Copyright © 2021, Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

Uncorrected Proof

2. Objectives

We aimed to compare the serum levels of cytokines and chemokines between mild and severe COVID-19 patients.

3. Methods

3.1 Study Population

In this study, a total of 78 patients admitted to the Hospital of Kafkas University, Kars, Eastern Turkey, and Çapa Hospital of Istanbul University, Western Turkey, were included. All subjects tested positive for SARS-CoV-2 in nasopharyngeal specimens by real-time reverse transcription polymerase chain reaction (RT-PCR). The patients were classified into two severe (n = 46) and mild (n = 32) groups according to disease severity definitions by the WHO (10). Demographic, clinical, laboratory, and radiological findings along with management and outcome data of patients were obtained from medical records.

3.2. Samples

After the diagnosis of COVID-19 using RT-PCR, blood samples were collected from all patients. The samples were centrifuged at 1000 xg for 15 minutes at 4°C followed by transfer of plasma to polypropylene tubes. Plasma samples were centrifuged again at 4000 xg for 15 minutes at 4°C to completely remove platelets and precipitates. Plasma samples were diluted fourfold (1: 4), aliquoted, and stored at -80°C until analysis.

3.3 Quantification of Cytokines, Chemokines, and Growth Factors

The serum levels of cytokines, chemokines, and growth factors were determined using the Bio-Plex Pro™ Human Cytokine Screening Panel (Bio-Rad, Hercules, CA, USA), which included the following 17 markers; G-CSF, granulocyte-macrophage (GM)- CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, MCP-1, MIP-1β, and TNF-α. serum samples, microbeads, standard curves, and reagents were prepared, and multiplex immunoassays were performed according to the manufacturer's protocol. Measurements were done using the BioPlex200 Multiplex System platform. The concentrations of cytokine and chemokines were calculated by reference to the standard curve.

3.4 Statistical Analysis

We described the continuous variables as mean $\pm$ standard deviation (SD) and the categorical variables as frequency rates and percentages. The normality assumption was tested by Shapiro-Wilk and Kolmogorov-Smirnov tests. The independent group t tests and Mann-Whitney U test were used to evaluate comparison of independent variables that were normally and non-normally distributed, respectively. Categorical variables were compared using the χ^2 test. Two-sided P-values less than 0.05 were considered statistically significant. All statistical analyses were performed using R version 3.6.0 program.

4. Results

Out of a total of 78 COVID-19 patients (54 males and 24 females) with confirmed COVID-19 infection, 46 (59%) patients were categorized as severe cases. The mean age was 43.1 $\pm$ 13.3 (range: 18 - 65) and 58.2 $\pm$ 15 (range: 35 - 92) years in mild and severe groups, respectively. Compared with mildly symptomatic patients, severe patients were significantly older and with a male predominance. The most common symptoms were myalgia or fatigue (67.9%) and cough (46.2%), followed by dyspnea (35.9%) and fever (34.6%). Comorbidities were present in more than half of the patients, with hypertension (33.3%) being the most common, followed by cardiovascular disease (14.1%) and diabetes (14.1%). Severe patients had significantly higher incidence of comorbid diseases. Also, eight patients (17.4%) were admitted to intensive care unit (ICU), of whom six case (75 %) had multiple comorbidities (Table 1).

We observed significant differences in hematological and biochemical laboratory findings between the two groups. While neutrophil and white blood cell (WBC) counts were significantly higher in severe patients, lymphocyte and platelet counts were lower in them. Compared with mild patients, severe patients had significantly higher levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), D-dimer, ferritin, C-reactive protein (CRP), and urea (Table 2). On admission, abnormalities on chest computed tomography (CT) were seen in all severe patients. Moreover, 42 (91.3%) patients had bilateral multilobar peripherally distributed ground glass opacities and consolidation areas, of whom 19 (41.3%) had more than 50% of infiltration of the lung parenchyma and four (8.7%) patients had few subpleural small ground glass opacities in one or two lobes.

All patients received favipiravir, as it is the only approved antiviral for COVID-19 patients in Turkey. Favipiravir was used for five days in mild patients and up to 10

Uncorrected Proof

Table 1. Demographics and Baseline Characteristics of Patients [a]

Variables	All (n = 78)	Severe (n = 46)	Mild (n = 32)	P-Value
Age (y)	51.7 ± 16.1 (18 - 92)	58 ± 15 (35 - 92)	43.1 ± 13.3 (18 - 65)	< 0.001
Sex				
Males	54 (69.2)	36 (78.3)	18 (56.2)	
Females	24 (30.8)	10 (21.7)	14 (43.8)	
Comorbid conditions				
Diabetes	11 (14.1)	10 (21.7)	1 (3.1)	0.021
Hypertension	26 (33.3)	24 (52.2)	2 (6.2)	< 0.001
Cardiovascular disease	11 (14.1)	11 (23.9)	-	0.002
COPD	7 (9)	7 (15.2)	-	0.037
Malignancy	1 (1.3)	1 (2.2)	-	1
Chronic liver disease	1 (1.3)	1 (2.2)	-	1
Signs and Symptoms				
Fever	27 (34.6)	27 (58.7)	-	< 0.001
Cough	36 (46.2)	31 (39.7)	5 (15.6)	< 0.001
Myalgia or fatigue	53 (67.9)	36 (78.3)	17 (53.1)	0.027
Headache	14 (17.9)	3 (6.5)	11 (34.4)	0.003
Diarrhea	4 (5.1)	3 (6.5)	1 (3.1)	0.64
Dyspnea	28 (35.9)	27 (58.7)	1 (3.1)	< 0.001
Nausea and vomiting	6 (7.7)	6 (13)	-	0.038
Sore throat	12 (15.4)	4 (8.7)	8 (25)	0.062

Abbreviation: COPD, chronic obstructive pulmonary disease.
[a] Values are expressed as No. (%) or mean ± SD (range).

days in severe patients who received supplemental oxygen support additionally. The nasal cannula (38, 82.6%) and non-invasive ventilation or high-flow nasal cannula (15, 32.6%) were the most common procedures applied to severe patients. Invasive mechanical ventilation was required in three (6.5%) patients, of whom two (66.6%) died. In severe patients needing oxygen therapy support due to respiratory distress, 6 mg/day dexamethasone or 0.5 - 1 mg/kg prednisolone was used for up to 10 days. In patients whose oxygen demand increased within 24 hours or whose acute phase response increased despite the treatment, a higher dose of corticosteroids was used after considering the risk factors of the patient. High-dose corticosteroids therapy was used on a case-by-case basis and in some cases for up to three days. After high-dose steroid administration, treatment with 6 mg/day dexamethasone was continued. Out of 46 severe patients, 35 (78.3%) received systematic corticosteroids, 36 (78.3%) antibiotic treatment, and 2 (4.3%) intravenous immunoglobulin therapy (Table 3).

The serum levels of 17 markers were evaluated; however, only TNF-α, IFN-γ, IL-6, IL-8, IL-13, MIP-1β, and MCP-1 levels could be analyzed between severe and mild patients (Table 3). The levels of G-CSF, GM-CSF, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12 (p70), IL-17, and IL-1β were not obtained due to low levels that could not be interpreted using standard curves. Therefore, these biomarkers were excluded from the final analysis. The median levels of IL-8 and IL-6 were significantly higher in severe patients than mild ones ($P < 0.001$ and $P < 0.001$, respectively). The median levels of IFN-γ, IL-13, MIP-1β, and MCP-1 were higher in severe patients, although the difference was not statistically significant ($P = 0.271$, $P = 0.119$, $P = 0.854$, and $P = 0.533$, respectively). In addition, there was no statistically significant difference between the two groups for TNF-α levels ($P = 0.644$).

5. Discussion

COVID-19 pandemic remains a global health problem with high rates of morbidity and substantial mortality. COVID-19 infection may be accompanied by an uncontrolled inflammatory response that triggers the overproduction of proinflammatory cytokines known as "cytokine

Uncorrected Proof

Table 2. Laboratory Findings of Patients

Laboratory Findings (Unit, Normal Range)	All (78); Mean ± SD	Severe (46); Mean ± SD	Mild (32); Mean ± SD	P-Value
White blood cell count ($\times 10^9$/L, 3.7 - 10.4)	6.8 ± 3.4	7.8 ± 3.7	5.4 ± 2.2	0.002
Neutrophil count ($\times 10^9$/L, 1.8 - 7.8)	5.1 ± 3.2	6.1 ± 3.7	3.7 ± 1.9	0.001
Lymphocyte count ($\times 10^9$/L, 0.9 - 3.7)	1.2 ± 0.6	1 ± 0.6	1.5 ± 0.6	< 0.001
Platelet count ($\times$ 109/L, 149 - 371)	240 ± 85.7	216.2 ± 52.6	257.4 ± 100.6	0.045
Prothrombin time (s, 9.7 - 13.9)	13.3 ± 3.7	13.1 ± 3.9	13.8 ± 2.9	0.549
Activated partial thromboplastin time (s, 22 - 45)	33.8 ± 11	33.4 ± 9.5	35.1 ± 14.6	0.616
Prothrombin time INR (0.8 - 1.24)	1,1 ± 0.2	1 ± 0.1	1.2 ± 0.2	0.002
D-dimer (ng/L, 0 - 500)	1.3 ± 2.8	1.8 ± 3.4	0.5 ± 0.7	0.042
Alanine aminotransferase (U/L, 0 - 41)	47.1 ± 47.9	57.5 ± 55.3	32.4 ± 29.9	0.03
Aspartate aminotransferase (U/L, 0 - 40)	44.7 ± 42.5	57.3 ± 50.9	26.6 ± 11.5	0.002
Creatine kinase (IU/L, 0 - 190)	142.1 ± 125.7	154.7 ± 137.3	108.5 ± 82.1	0.228
Lactate dehydrogenase (U/L, 135 - 225)	305.8 ± 154.9	373.3 ± 165.3	203.3 ± 40.6	< 0.001
Total bilirubin (mg/dL, 0 - 1.2)	0.6 ± 0.3	0.6 ± 0.3	0.5 ± 0.2	0.3
Potassium (mmol/L,.5 - 5.1)	6.1 ± 15.9	7.5 ± 20.8	4.1 ± 0.4	0.384
Sodium (mmol/L, 136 - 145)	135,4 ± 16.7	132.6 ± 20.9	139.7 ± 3.1	0.089
Urea (mg/dL, 10 - 50)	41.5 ± 21.7	46.7 ± 24.7	33.3 ± 12.6	0.013
Creatinine (mg/dL, 0.7 - 1.2)	1.1 ± 0.6	1.1 ± 0.7	1 ± 0.3	0.323
Ferritin (ng/mL, 30 - 400)	844 ± 870.3	1303.3 ± 860.4	171.4 ± 147.3	< 0.001
C-reactive protein (mg/dL, 0 - 0.5)	52.1 ± 84.1	139.2 ± 84.1	1 ± 2.6	< 0.001

Table 3. The Serum Levels of Proinflammatory Cytokines and Chemokines in Patients

Immune Mediator	Severe (n = 46)		Mild (n = 32)		P-Value [a]
	Mean ± SD	[Q1:Q3] Median	Mean ± SD	[Q1:Q3] Median	
IL-6, pg/mL	60.3 ± 95.3	[19.50:91.98] 29.6	4.3 ± 2.4	[3.08:5.79] 3.46	< 0.001
TNF-α, pg/mL	48 ± 53.9	[19.28:66.00] 35.94	30.7 ± 11.6	[29.34:45.85] 35.98	0.644
IFN-γ, pg/mL	14.3 ± 26.3	[3.71:11.49] 5.82	6.5 ± 7.1	[3.01:5.47] 3.01	0.271
IL-13, pg/mL	1.6 ± 0.7	[1.14:1.63] 1.63	1.3 ± 0.3	[1.14:1.14] 1.14	0.119
MCP-1, pg/mL	159.5 ± 262.8	[91.92:286.97] 107.35	86,4 ± 51.7	[65.32:126.36] 99.03	0.533
IL-8, pg/mL	59 ± 154.9	[15.96:51.06] 35.64	12 ± 5	[13.13:17.38] 15.48	< 0.001
MIP-1β, pg/mL	54.1 ± 89.2	[29,51:75,93] 53	38.6 ± 15.6	[41,07:53,33] 43.96	0.854

[a] Statistical analysis was performed using median values.

storm" (6, 11). The increase in circulating proinflammatory cytokines, including TNF-α, IFN-γ, IL-6, IL-8, and IL-2 results in cytokine storm, which is thought to be associated with disease severity (12, 13). Therefore, recent studies have focused on determining the association between inflammatory responses and severity of COVID-19. Our findings showed that the main clinical symptoms of severe COVID-19 patients were myalgia, fatigue, cough, dyspnea, and fever. We demonstrated that severe patients were significantly older than mild patients, and they had comorbidities such as hypertension, cardiovascular disease, and diabetes. Severe patients were characterized by significant laboratory abnormalities such as increased WBC and neutrophil counts and decreased lymphocyte and platelet counts. The levels of ALT, AST, LDH, urea, ferritin, D-dimer, and CRP were higher in severe patients. Also, we found that IL-6 and IL-8 significantly increased in severe patients. Accordingly, older age, presence of comorbid conditions, hematological and biochemical laboratory abnormalities, and higher levels of inflammatory cytokines were closely

Uncorrected Proof

related to disease progression of COVID-19, which is consistent with the results of some previous reports (14).

To date, it has not been explained why some infected patients are asymptomatic, while others have a range of mild, moderate, severe, and critical symptoms. Previous studies suggested that an uncontrolled and overproduction of proinflammatory cytokines/chemokines by immune system might play a major role in severity of MERS-CoV, SARS-CoV, and SARS-CoV-2 infections. The elevated levels of proinflammatory cytokines/chemokines, such as IL-6, IL-8, IFN-γ, and TNF-α were found to be associated with disease severity or mortality in COVID-19 patients, which is similar to SARS-CoV and MERS-CoV infections (15-20). Previous studies have shown that marked elevation of proinflammatory cytokines, such as IL-1, TNF-α, IL-6, TGF-β, and IL-10 were correlated with pulmonary inflammation and severe lung impairment in MERS-CoV patients (20). SARS-CoV infection was also reported to induce ARDS associated with highly expressed proinflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-12, INF-γ, and TNF-α (21-23).

In our study, the serum levels of IL-6, IL-8, IL-13, IFN-γ, MIP-1β, and MCP-1 increased in the severe group compared to the mild group; however, statistically significant differences were observed only in IL-6 and IL-8 levels. Gong et al. demonstrated that higher levels of IL-2R, IL-6, IL-8, IL-10, and TNF-α were associated with COVID-19 progression (24). Karki et al. suggested that serum levels of IL-6 and TNF-α were significant predictors of disease severity and death in COVID-19 infection (25). In addition, a meta-analysis by Akbari et al., including 7,865 COVID-19 patients, showed significant increases in IL-2, IL-2 receptor (IL-2R), IL-4, IL-6, IL-8, IL-10, TNF-α, and INF-γ in the severe patients compared to the non-severe patients (26). Most clinical studies reported that severe COVID-19 patients had significantly higher levels of circulating proinflammatory cytokines/chemokines, including IL-2, IL-2R, IL-6, IL-8, IL-10, and TNF-α, than mild patients (Table 4) (12, 19, 20, 27-33). Our results are consistent with other studies, suggesting that the higher levels of IL-6 and IL-8 are associated with the disease severity.

IL-6 is a well-known proinflammatory cytokine induced by endothelial cells myeloid cells, smooth muscle cells, and T cells, and it takes part in different signal transduction pathways (34). The binding of the SARS-COV-2 spike protein to ACE (angiotensin-converting enzyme)-2 as its receptor results in activation of signal transduction pathways leading to production of IL-6 (35). The increased level of IL-6 was found to be associated with ICU admission, ARDS, and death in COVID-19 infection (36). Several studies showed that serum IL-6 levels had a potential prognostic value for need of mechanical ventilation, severity of disease, and mortality in COVID-19 (37). There is insufficient knowledge about the role of chemokines in immunopathogenesis of COVID-19 compared to cytokines, even though they are being reported increasingly. Previous studies showed that upregulated chemokines, including CXCL8, CXCL10, and CCL2 were correlated with disease severity and increased mortality in COVID-19 patients (38). CXCL8, also known as IL-8, is a potent chemoattractant that recruits and activates neutrophils in the lungs during inflammation, and it may play a major role in development of ARDS in COVID-19 patients (39, 40). The high level of IL-8 has been demonstrated to correlate with increased amounts of neutrophils in the peripheral blood and infiltration of neutrophils into the lungs during COVID-19 infection. Also, Ma et al. reported that the high levels of circulating IL-8 were associated with duration of illness in severe COVID-19 patients (41).

The determination of association between severity of COVID-19 and IL-6 and IL-8 levels provides supportive approach to ongoing clinical trials and studies that are targeting cytokine pathways, such as IL-6 inhibitors like tocilizumab (TCZ), sarilumab, and siltuximab or IL-8 inhibitors like BMS-986253 and reparixin (42-44). Also, interfering with the production of and reducing the levels of these cytokines/chemokines seems to be a promising immunomodulation strategy to control the infection-associated hyperinflammation in severe COVID-19 cases. Xu et al. observed that the symptoms, laboratory abnormalities, and CT opacity changes improved, but oxygen requirement reduced in all patients treated with TCZ (45). A meta-analysis including 3,641 patients from 16 studies showed that TCZ might reduce mortality in severe patients (46). However, a significant problem that needs to be answered is which therapeutic blockade for COVID-19 treatment should be used since a variety of cytokines and chemokines have been reported to be increased in several studies comparing severe and non-severe cases. Hence, further studies are needed to explore the role of specific immunomodulation with cytokine/chemokine antagonists.

This study had several limitations. First, we could not determine the kinetic changes of inflammatory response in disease progression, as sequential serum samples were not available. Second, we could evaluate only seven biomarkers, although our study was designed to investigate the extensive plasma cytokine and chemokine profiles of severe and mild COVID-19 patients due to low levels that could not be interpreted using standard curves. More extensive studies of cytokine and chemokine profiles may help to understand the immunological mechanisms of COVID-19.

Uncorrected Proof

Table 4. The Increased Cytokine Patterns Related to Severe Patients of COVID-19

Author and Year (Reference)	Country	Groups	Cases	Age	Sex (male, %)	Methods	Cytokines	Ref. No.
Liu et al. 2020	China	Mild	27	43.2 ± 12.3	8 (29.6)	Flow cytometry	IL-6, IL-10, IL-2 and IFN-γ	(20)
		Severe	13	59.7 ± 10.1	7 (53.8)			
Liu et al. 2020	China	Mild	46	NA	NA	NA	IL-2R, IL-6, and IL-8	(27)
		Severe	30	NA	NA			
Zhang et al. 2020	China	Mild	29	44.34 ± 15.84	17 (58.6)	Flow cytometry	IL-6 and IL-10	(28)
		Severe	14	61.7 ± 9.22	5 (35.7)			
Wan et al. 2020	China	Mild	102	43.05 ± 13.12	55 (53.9)	NA	IL-6 and IL-10	(29)
		Severe	21	61.29 ± 15.55	11 (52.4)			
Tan et al. 2020	China	Mild/moderate	31	44.5	17 (53.1)	Flow cytometry	IL-2 and IL-6, IL-10 and TNF-α	(30)
		Severe	25	66	18(72)			
Chen et al. 2020	China	Moderate	10	52	7 (70.0)	NA	IL-2R, IL-6, IL-10, and TNF-α	(31)
		Severe	11	61	10 (90.9)			
Li et al. 2020	China	Non-severe	279	56	126 (45.2)	NA	IL-2R, IL-6, IL-10, and TNF-α.	(32)
		Severe	269	65	153 (56.9)			
Qin et al. 2020	China	Non-severe	166	53	80 (48.2)	NA	IL-2R, IL-6, IL-8, IL-10, and TNF-α	(19)
		Severe	286	61	155 (54.2)			
Yang et al. 2020	China	Non-severe	69	42.1 ± 18.6	38 (55.1)	Flow cytometry	IL-2R, IL-6, IL-8 and IL-10	(12)
		Severe	24	57.9 ± 11.8	18 (75)			
Huang et al. 2020	China	ICU	13	49	NA	Multiplex Immunoassay	IL-2, IL-7, IL-10, GCSF, IP-10, MCP1, MIP1A and TNF-α	(33)
		Non-ICU	28	49	NA			
Our study	Turkey	Mild	32	43,1 ± 13,3	18 (56.2)	Multiplex Immunoassay	IL-6 and IL-8	
		Severe	46	58 ± 15	36 (78.3)			

5.1. Conclusions

This is the first clinical study from Turkey descriptively evaluating the cytokine and chemokine levels in COVID-19 patients. Our results demonstrated that IL-6 and IL-8 levels were higher in severe patients. Also, the levels of IL-6 and IL-8 can be used as potential prognostic biomarkers of disease severity in COVID-19 patients. Therefore, detection of plasma cytokine and chemokine levels may be an indicator of disease progression and provide alternative approaches for immunomodulation of severe COVID-19 cases to control the infection-associated hyperinflammation.

Footnotes

Authors' Contribution: Study concept and design, D.O., E.T., M.K., A.M.; Acquisition of data, D.O., E.T., M.K., A.M., N.S., O.A.; Analysis and interpretation of data, D.O., E.T., A.M.; Drafting of the manuscript, D.O., E.T., M.K., A.M., N.S., O.A.; Critical revision of the manuscript for important intellectual content, D.O., E.T.; Statistical analysis, D.O., E.T.; Administrative, technical, and material support, D.O., M.K.; Study supervision. D.O., E.T.

Conflict of Interests: The authors have no conflict of interest.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

Ethical Approval: This study was approved by the Republic of Turkey Ministry of Health COVID-19 Scientific Research Evaluation Commission (Approval date: 07/05/2020; number: 2020-05-04T18-11-34) and Ethics Commission of the Faculty of Medicine, Kafkas University (Ap-

Uncorrected Proof

proval date: 13/05/2020 number: 80576354-050-99/150).

Funding/Support: The current study was supported by the Scientific Research Project Council of Kafkas University, Kars, Turkey (project number: 2020-TS-26).

References

1. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the "Cytokine Storm" in COVID-19. *J Infect*. 2020;**80**(6):607–13. doi: 10.1016/j.jinf.2020.03.037. [PubMed: 32283152]. [PubMed Central: PMC7194613].
2. Schildgen O. The human Coronaviruses. In: Tang YW, Stratton CW, editors. *Advanced techniques in diagnostic microbiology*. 3rd ed. New York, USA: Springer, Cham; 2018. p. 269–80. doi: 10.1007/978-3-319-95111-9_11.
3. Silverman AI, Boehm AB. Systematic review and meta-analysis of the persistence and disinfection of human coronaviruses and their viral surrogates in water and wastewater. *Environ Sci Tech Let*. 2020;**7**(8):544–53. doi: 10.1021/acs.estlett.0c00313.
4. Singhal T. A review of Coronavirus disease-2019 (COVID-19). *Indian J Pediatr*. 2020;**87**(4):281–6. doi: 10.1007/s12098-020-03263-6. [PubMed: 32166607]. [PubMed Central: PMC7090728].
5. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet*. 2020;**395**(10223):507–13. doi: 10.1016/S0140-6736(20)30211-7. [PubMed: 32007143]. [PubMed Central: PMC7135076].
6. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; what we know so far. *Front Immunol*. 2020;**11**:1446. doi: 10.3389/fimmu.2020.01446. [PubMed: 32612617]. [PubMed Central: PMC7308649].
7. Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. *J Mol Histol*. 2020;**51**(6):613–28. doi: 10.1007/s10735-020-09915-3. [PubMed: 33011887]. [PubMed Central: PMC7533045].
8. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72314 cases from the Chinese center for disease control and prevention. *JAMA*. 2020;**323**(13):1239–42. doi: 10.1001/jama.2020.2648. [PubMed: 32091533].
9. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. *J Med Virol*. 2021;**93**(1):250–6. doi: 10.1002/jmv.26232. [PubMed: 32592501]. [PubMed Central: PMC7361342].
10. National Institutes of Health. *Coronavirus disease 2019 (COVID-19) treatment guidelines*. Maryland , USA: National Institutes of Health; 2021, [cited 2021]. Available from: https://www.covid19treatmentguidelines.nih.gov/.
11. Song P, Li W, Xie J, Hou Y, You C. Cytokine storm induced by SARS-CoV-2. *Clin Chim Acta*. 2020;**509**:280–7. doi: 10.1016/j.cca.2020.06.017. [PubMed: 32531256]. [PubMed Central: PMC7283076].
12. Yang AP, Li HM, Tao WQ, Yang XJ, Wang M, Yang WJ, et al. Infection with SARS-CoV-2 causes abnormal laboratory results of multiple organs in patients. *Aging (Albany NY)*. 2020;**12**(11):10059–69. doi: 10.18632/aging.103255. [PubMed: 32484453]. [PubMed Central: PMC7346014].
13. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med*. 2020;**26**(10):1636–43. doi: 10.1038/s41591-020-1051-9. [PubMed: 32839624]. [PubMed Central: PMC7869028].
14. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *Int J Infect Dis*. 2020;**96**:467–74. doi: 10.1016/j.ijid.2020.05.055. [PubMed: 32425643]. [PubMed Central: PMC7233226].
15. Chien JY, Hsueh PR, Cheng WC, Yu CJ, Yang PC. Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. *Respirology*. 2006;**11**(6):715–22. doi: 10.1111/j.1440-1843.2006.00942.x. [PubMed: 17052299]. [PubMed Central: PMC7192207].
16. Chu H, Zhou J, Wong BH, Li C, Cheng ZS, Lin X, et al. Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. *Virology*. 2014;**454-455**:197–205. doi: 10.1016/j.virol.2014.02.018. [PubMed: 24725946]. [PubMed Central: PMC7111975].
17. Zhou J, Chu H, Li C, Wong BH, Cheng ZS, Poon VK, et al. Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. *J Infect Dis*. 2014;**209**(9):1331–42. doi: 10.1093/infdis/jit504. [PubMed: 24065148]. [PubMed Central: PMC7107356].
18. Kong SL, Chui P, Lim B, Salto-Tellez M. Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients. *Virus Res*. 2009;**145**(2):260–9. doi: 10.1016/j.virusres.2009.07.014. [PubMed: 19635508]. [PubMed Central: PMC7114434].
19. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis*. 2020;**71**(15):762–8. doi: 10.1093/cid/ciaa248. [PubMed: 32161940]. [PubMed Central: PMC7108125].
20. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine*. 2020;**55**:102763. doi: 10.1016/j.ebiom.2020.102763. [PubMed: 32361250]. [PubMed Central: PMC7165294].
21. Lam CW, Chan MH, Wong CK. Severe acute respiratory syndrome: Clinical and laboratory manifestations. *Clin Biochem Rev*. 2004;**25**(2):121–32. [PubMed: 18458712]. [PubMed Central: PMC1904416].
22. Sheng WH, Chiang BL, Chang SC, Ho HN, Wang JT, Chen YC, et al. Clinical manifestations and inflammatory cytokine responses in patients with severe acute respiratory syndrome. *J Formos Med Assoc*. 2005;**104**(10):715–23. [PubMed: 16385373].
23. Zhu M. SARS Immunity and Vaccination. *Cell Mol Immunol*. 2004;**1**(3):193–8. [PubMed: 16219167].
24. Gong J, Dong H, Xia QS, Huang ZY, Wang DK, Zhao Y, et al. Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: A retrospective study. *BMC Infect Dis*. 2020;**20**(1):963. doi: 10.1186/s12879-020-05681-5. [PubMed: 33349241]. [PubMed Central: PMC7750784].
25. Karki R, Sharma BR, Tuladhar S, Williams EP, Zalduondo L, Samir P, et al. Synergism of TNF-alpha and IFN-gamma triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes. *Cell*. 2021;**184**(1):149–168 e17. doi: 10.1016/j.cell.2020.11.025. [PubMed: 33278357]. [PubMed Central: PMC7674074].
26. Akbari H, Tabrizi R, Lankarani KB, Aria H, Vakili S, Asadian F, et al. The role of cytokine profile and lymphocyte subsets in the severity of Coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *Life Sci*. 2020;**258**:118167. doi: 10.1016/j.lfs.2020.118167. [PubMed: 32735885]. [PubMed Central: PMC7387997].
27. Liu Y, Liao W, Wan L, Xiang T, Zhang W. Correlation between relative nasopharyngeal virus RNA load and lymphocyte count disease severity in patients with COVID-19. *Viral Immunol*. 2021;**34**(5):330–5. doi: 10.1089/vim.2020.0062. [PubMed: 32297828].
28. Zhang H, Wang X, Fu Z, Luo M, Zhang Z, Zhang K, et al. Potential factors for prediction of disease severity of COVID-19 patients. *MedRxiv*. 2020;**Preprint**. doi: 10.1101/2020.03.20.20039818.

Uncorrected Proof

29. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). *MedRxiv*. 2020;**Preprint**. doi: 10.1101/2020.02.10.20021832.
30. Tan M, Liu Y, Zhou R, Deng X, Li F, Liang K, et al. Immunopathological characteristics of Coronavirus disease 2019 cases in Guangzhou, China. *Immunology*. 2020;**160**(3):261–8. doi: 10.1111/imm.13223. [PubMed: 32460357]. [PubMed Central: PMC7283723].
31. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate Coronavirus disease 2019. *J Clin Invest*. 2020;**130**(5):2620–9. doi: 10.1172/JCI137244. [PubMed: 32217835]. [PubMed Central: PMC7190990].
32. 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**(1):110–8. doi: 10.1016/j.jaci.2020.04.006. [PubMed: 32294485]. [PubMed Central: PMC7152876].
33. 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**(10223):497–506. doi: 10.1016/S0140-6736(20)30183-5. [PubMed: 31986264]. [PubMed Central: PMC7159299].
34. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;**6**(10). a016295. doi: 10.1101/cshperspect.a016295. [PubMed: 25190079]. [PubMed Central: PMC4176007].
35. Iwasaki M, Saito J, Zhao H, Sakamoto A, Hirota K, Ma D. Inflammation triggered by SARS-CoV-2 and ACE2 augment drives multiple organ failure of severe COVID-19: Molecular mechanisms and implications. *Inflammation*. 2021;**44**(1):13–34. doi: 10.1007/s10753-020-01337-3. [PubMed: 33029758]. [PubMed Central: PMC7541099].
36. Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: A systematic review and meta-analysis. *Rev Med Virol*. 2020;**30**(6):1–9. doi: 10.1002/rmv.2141. [PubMed: 32845568]. [PubMed Central: PMC7460877].
37. Santa Cruz A, Mendes-Frias A, Oliveira AI, Dias L, Matos AR, Carvalho A, et al. Interleukin-6 is a biomarker for the development of fatal severe acute respiratory syndrome Coronavirus 2 pneumonia. *Front Immunol*. 2021;**12**:613422. doi: 10.3389/fimmu.2021.613422. [PubMed: 33679753]. [PubMed Central: PMC7930905].
38. Khalil BA, Elemam NM, Maghazachi AA. Chemokines and chemokine receptors during COVID-19 infection. *Comput Struct Biotechnol J*. 2021;**19**:976–88. doi: 10.1016/j.csbj.2021.01.034. [PubMed: 33558827]. [PubMed Central: PMC7859556].
39. Mulchandani R, Lyngdoh T, Kakkar AK. Deciphering the COVID-19 cytokine storm: Systematic review and meta-analysis. *Eur J Clin Invest*. 2021;**51**(1). e13429. doi: 10.1111/eci.13429. [PubMed: 33058143]. [PubMed Central: PMC7646004].
40. 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. doi: 10.1016/j.cytogfr.2020.05.003. [PubMed: 32446778]. [PubMed Central: PMC7211650].
41. Ma A, Zhang L, Ye X, Chen J, Yu J, Zhuang L, et al. High levels of circulating IL-8 and soluble IL-2R are associated with prolonged illness in patients with severe COVID-19. *Front Immunol*. 2021;**12**:626235. doi: 10.3389/fimmu.2021.626235. [PubMed: 33584733]. [PubMed Central: PMC7878368].
42. Koenig LM, Boehmer DFR, Metzger P, Schnurr M, Endres S, Rothenfusser S. Blocking inflammation on the way: Rationale for CXCR2 antagonists for the treatment of COVID-19. *J Exp Med*. 2020;**217**(9). doi: 10.1084/jem.20201342. [PubMed: 32678432]. [PubMed Central: PMC7365736].
43. Atal S, Fatima Z. IL-6 Inhibitors in the treatment of serious COVID-19: A promising therapy? *Pharmaceut Med*. 2020;**34**(4):223–31. doi: 10.1007/s40290-020-00342-z. [PubMed: 32535732]. [PubMed Central: PMC7292936].
44. Gonzalez-Aparicio M, Alfaro C. Significance of the IL-8 pathway for immunotherapy. *Hum Vaccin Immunother*. 2020;**16**(10):2312–7. doi: 10.1080/21645515.2019.1696075. [PubMed: 31860375]. [PubMed Central: PMC7644160].
45. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci USA*. 2020;**117**(20):10970–5. doi: 10.1073/pnas.2005615117. [PubMed: 32350134]. [PubMed Central: PMC7245089].
46. Boregowda U, Perisetti A, Nanjappa A, Gajendran M, Kutti Sridharan G, Goyal H. Addition of tocilizumab to the standard of care reduces mortality in severe COVID-19: A systematic review and meta-analysis. *Front Med (Lausanne)*. 2020;**7**:586221. doi: 10.3389/fmed.2020.586221. [PubMed: 33123544]. [PubMed Central: PMC7566918].