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Research Article

Cytokine and Chemokine Profiles in COVID-19: Why Did Conventional Inflammatory Markers Outperform Cytokines?

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Abstract

Objectives: Dysregulated inflammation drives severe COVID-19. Cytokine profiling has been proposed for risk stratification. Its value over conventional markers is unclear. We compared admission cytokine and chemokine levels in mild and severe COVID-19. We also examined why routine markers may outperform cytokines.

Methods: This single-center cross-sectional study enrolled 78 adults with confirmed COVID-19. Patients were classified as mild or severe using WHO criteria at emergency admission. Serum IL-13, TNF-alpha, IFN-gamma, macrophage inflammatory protein-1 beta, and monocyte chemoattractant protein-1 were measured. Demographics, routine laboratory markers, and cytokines were compared between groups.

Results: Thirty-two patients had mild disease and 46 had severe disease. Cytokine and chemokine levels were numerically higher in severe disease. None of these differences were statistically significant.

Conclusion: Conventional inflammatory and tissue-injury markers tracked severity better than single-time-point cytokine measurements. Standalone cytokine profiling at admission may offer limited value. Larger studies with serial sampling, broader panels, and adjustment for confounders are needed.

Keywords: COVID-19, cytokines, chemokines, disease severity, MCP-1, MIP-1β

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Coronavirus disease 2019 (COVID-19) is a heterogeneous infectious disease ranging from asymptomatic or mild upper respiratory tract involvement to severe viral pneumonia, acute respiratory distress syndrome, thromboembolic complications, multiorgan dysfunction, and death.[1,2] Although direct viral cytopathic injury contributes to early disease pathogenesis, the clinical deterioration observed in severe COVID-19 is largely shaped by dysregulated host inflammation, endothelial injury, coagulation activation, and tissue damage.[3,4] This inflammatory phenotype has led to intense interest in cytokines and chemokines as potential biomarkers of disease severity and therapeutic targets.

Early reports of severe COVID-19 described increased circulating concentrations of several inflammatory mediators, including interleukin-6, interleukin-8, interleukin-10, tumor necrosis factor-alpha, interferon-gamma-related pathways, monocyte chemoattractant protein-1, and macrophage inflammatory proteins.[5,6] These findings supported the concept that a cytokine-driven hyperinflammatory response may contribute to respiratory failure and systemic complications in a subgroup of patients. Subsequent studies and reviews, however, have shown that cytokine profiles in COVID-19 are not uniform and may vary according to disease phase, timing of sampling, assay platform,

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viral variant, age, comorbidities, and prior or ongoing anti-inflammatory treatment.[7,8]

In routine clinical practice, conventional inflammatory and tissue injury markers, such as C-reactive protein, ferritin, lactate dehydrogenase, D-dimer, leukocyte and neutrophil counts, lymphocyte count, and platelet count, have remained more accessible and more consistently associated with severe disease than specialized cytokine measurements. Several studies have reported that elevated CRP, ferritin, LDH, D-dimer, and neutrophil-to-lymphocyte ratio are associated with worse clinical outcomes, while lymphopenia and thrombocytopenia reflect immune dysregulation and disease severity.[9-12]

Despite the biological plausibility of cytokine profiling, its incremental value over conventional laboratory markers remains uncertain, particularly when measured at a single time point at hospital admission. In this context, negative or nonsignificant cytokine findings are clinically informative, as they may indicate that isolated baseline cytokine measurements do not sufficiently capture the dynamic inflammatory trajectory of COVID-19. This is especially relevant for cytokines and chemokines such as interleukin-13, tumor necrosis factor-alpha, interferon-gamma, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 beta, which may show biological elevation without providing robust discrimination between severity groups.

Therefore, this study aimed to compare admission serum cytokine and chemokine levels between patients with mild and severe COVID-19 and to evaluate their relationship with conventional inflammatory and tissue injury markers. We specifically examined whether selected serum cytokines and chemokines measured at emergency department admission could distinguish severe disease and why conventional laboratory markers may have outperformed cytokine measurements in reflecting COVID-19 severity. The study included 78 patients with COVID-19, classified as 32 mild and 46 severe cases according to World Health Organization disease severity criteria.[13] Cytokine and chemokine measurements were performed using the Bio-Plex Pro Human Cytokine Screening Panel.

Materials and Methods

Study Design and Setting

This was a single-center, cross-sectional observational study conducted at İstanbul University, Faculty of Medicine, between January 2022 and March 2022. The study included adult patients who presented to the emergency department with suspected COVID-19 and had confirmed SARS-CoV-2 infection by real-time reverse transcription polymerase chain reaction from combined nasopharyngeal and oropharyngeal swab samples. Patients were enrolled at hospital admission, and blood samples for cytokine and chemokine analyses were obtained before the initiation of COVID-19-specific treatment.

The study was designed to evaluate whether pretreatment serum cytokine and chemokine levels measured at admission were associated with COVID-19 disease severity and to compare their performance with conventional inflammatory and tissue injury markers.

Study Population

Patients aged 18 years or older with confirmed COVID-19 were eligible for inclusion. A total of 78 patients were included in the final analysis. At the time of emergency department admission, patients were evaluated according to clinical findings, laboratory parameters, and radiological features. Based on the World Health Organization COVID-19 disease severity classification, patients were categorized into two groups: mild disease and severe disease. Accordingly, 32 patients were classified as having mild COVID-19 and 46 patients as having severe COVID-19.

For all included patients, routine laboratory tests were obtained as part of clinical care, and an additional 10 mL blood sample was collected for cytokine and chemokine measurement.

Disease Severity Classification

COVID-19 disease severity was determined according to the World Health Organization classification. Patients without evidence of viral pneumonia or hypoxia were classified as having mild disease. Patients with clinical, radiological, or laboratory findings consistent with severe COVID-19 were classified as having severe disease. Classification was based on the overall assessment at emergency department admission, including clinical status, oxygenation, laboratory findings, and radiological involvement.

Data Collection

Demographic, clinical, laboratory, and radiological data were collected at the time of admission. The following baseline variables were recorded:

- age
- sex
- clinical presentation
- disease severity category
- routine hematological parameters
- biochemical markers
- inflammatory markers
- coagulation-related markers

Laboratory parameters included leukocyte count, neutrophil count, lymphocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, urea, ferritin, D-dimer, and C-reactive protein. These parameters were compared between the mild and severe disease groups.

Cytokine and Chemokine Measurement

Blood samples for cytokine and chemokine analysis were obtained at emergency department admission before the initiation of COVID-19-specific treatment. This timing was chosen to minimize the potential confounding effects of corticosteroids, antiviral agents, anticoagulants, or other anti-inflammatory treatments on circulating cytokine and chemokine concentrations.

Samples were centrifuged at 1000×g for 15 minutes at 4°C. The plasma fraction was then transferred into polypropylene tubes. To remove residual platelets and precipitates, plasma samples were centrifuged again at 4000×g for 15 minutes at 4°C. The samples were diluted at a 1:4 ratio and stored at –80°C until analysis.

Serum cytokine and chemokine levels were measured using the Bio-Plex Pro Human Cytokine Screening Panel (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The measured mediators included tumor necrosis factor-alpha, interferon-gamma, interleukin-13, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 beta. Measurements were performed using the Bio-Plex 200 multiplex system platform.

Outcomes

The primary outcome was the association between pretreatment admission serum cytokine and chemokine levels and COVID-19 disease severity, defined according to World Health Organization severity criteria.

The secondary outcome was the association between conventional laboratory markers and COVID-19 disease severity. Conventional markers included leukocyte count, neutrophil count, lymphocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, urea, ferritin, D-dimer, and C-reactive protein.

Data on intensive care unit admission, mortality, mechanical ventilation, or detailed oxygen requirement were not available; therefore, these endpoints were not analyzed.

Statistical Analysis

All statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables were presented as median (IQR) and n (%), respectively. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the assumption of normality. Independent samples t-tests and Mann-Whitney U tests were used to compare normally distributed and nonnormally distributed independent variables, respectively. Categorical variables were compared using the chi-square test. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University, Türkiye, with approval dated December 31, 2021, and approval number 671545. Written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Study Population and Demographic Characteristics

A total of 78 patients with confirmed COVID-19 were included in the study. Of these, 54 patients were male and 24 were female. According to the World Health Organization COVID-19 disease severity classification, 46 patients were classified as having severe disease and 32 patients as having mild disease.

The overall mean age was 51.7±16.1 years. Patients with severe COVID-19 were significantly older than those with mild disease, with mean ages of 58.2±15.0 years and 43.1±13.3 years, respectively ($p<0.001$). Male sex was more frequent in the severe disease group than in the mild disease group (36/46 patients [78.3%] vs. 18/32 patients [56.2%], $p=0.048$).

Comorbidities were more common among patients with severe disease. Diabetes mellitus was present in 10 patients with severe disease and in 1 patient with mild disease (21.7% vs. 3.1%, $p=0.021$). Hypertension was also significantly more frequent in severe disease (24 patients [52.2%] vs. 2 patients [6.2%], $p<0.001$). Cardiovascular disease and chronic obstructive pulmonary disease were observed only in the severe disease group (23.9% and 15.2%, respectively), and both were significantly associated with severe disease. The demographic characteristics of patients with mild and severe COVID-19 are presented in Table 1.

Clinical Characteristics

The most common symptoms in the overall cohort were myalgia or fatigue (53 patients [67.9%]), cough (36 patients [46.3%]), dyspnea (28 patients [35.9%]), and fever (27 patients [34.6%]).

Fever was observed only in the severe disease group (27 patients [58.7%] vs. 0 patients in the mild disease group, $p<0.001$).

Table 1. Demographic characteristics of patients with mild and severe COVID-19

	All (n=78)	Severe (n=46)	Mild (n=32)	p
Age	51.7±16.1 (18-92)	58±15 (35-92)	43.1±13.3 (18-65)	**<0.001**
Sex				
Male	54 (69.2%)	36 (78.3%)	18 (56.2%)	0.048
Female	24 (30.8%)	10 (21.7%)	14 (43.8%)	
Comorbidity				
DM	11 (14.1%)	10 (21.7%)	1 (3.1%)	0.021
HT	26 (33.3%)	24 (52.2%)	2 (6.2%)	**<0.001**
CardiovascularyDisease	11 (14.1%)	11 (23.9%)	-	0.002
COPH	7 (9%)	7 (15.2%)	-	0.037
Malignancy	1 (1.3%)	1 (2.2%)	-	1
Chronic Hepatic Disease	1 (1.3%)	1 (2.2%)	-	1

Cough was also significantly more frequent in severe disease (31 patients [67.4%] vs. 5 patients [15.6%], p<0.001). Dyspnea showed one of the clearest differences between groups, being present in 27 patients with severe disease (58.7%) and in only 1 patient with mild disease (3.1%) (p<0.001).

Myalgia or fatigue was more common in the severe disease group than in the mild disease group (36 patients [78.3%] vs. 17 patients [53.1%], p=0.027). Headache was more frequent in the mild disease group (11 patients [34.4%] vs. 3 patients [6.5%], p=0.003). Nausea and vomiting were observed only in the severe disease group (6 patients [13.0%], p=0.038). Diarrhea and sore throat did not differ significantly between groups. The clinical characteristics of patients according to COVID-19 disease severity are summarized in Table 2.

Conventional Laboratory Markers According to Disease Severity

Several conventional laboratory markers differed significantly between the mild and severe disease groups. Patients with severe COVID-19 had significantly higher leukocyte counts than patients with mild disease [7.8±3.7 vs. 5.4±2.2×10^9/L, p=0.002]. Neutrophil counts were also significantly higher in severe disease [6.1±3.7 vs. 3.7±1.9×10^9/L, p=0.001], whereas lymphocyte counts were significantly lower [1.0±0.6 vs. 1.5±0.6×10^9/L, p<0.001].

Platelet counts were lower in severe disease than in mild disease [216.2±52.6 vs. 257.4±100.6×10^9/L, p=0.045]. INR was also significantly different between groups [1.0±0.1 vs. 1.2±0.2, p=0.002]. D-dimer levels were higher in severe disease [1.8±3.4 vs. 0.5±0.7 ng/L, p=0.042].

Markers of liver injury and tissue damage were also more elevated in severe disease. Alanine aminotransferase was higher in severe disease [57.5±55.3 vs. 32.4±29.9 U/L, p=0.030], as was aspartate aminotransferase [57.3±50.9 vs. 26.6±11.5 U/L, p=0.002]. Lactate dehydrogenase showed a marked increase in severe disease [373.3±165.3 vs. 203.3±40.6 U/L, p<0.001].

Inflammatory markers showed the strongest differences between groups. Ferritin levels were substantially higher in pa-

Table 2. Clinical characteristics of patients according to COVID-19 disease severity

Symptoms and Signs	All (n=78)	Severe (n=46)	Mild (n=32)	p
Fewer	27 (34.6%)	27 (58.7%)	-	**<0.001**
Cough	36 (46.3%)	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
Dispnea	28 (35.9%)	27 (58.7%)	1 (3.1%)	**<0.001**
Neausea and vomitting	6 (7.7%)	6 (13%)	-	**0.038**
Sore Throat	12 (15.4%)	4 (8.7%)	8 (25%)	0.062

tients with severe COVID-19 [1303.3±860.4 vs. 171.4±147.3 ng/mL, p<0.001]. C-reactive protein levels were also markedly higher in the severe disease group [139.2±84.1 vs. 1.0±2.6 mg/dL, p<0.001]. Urea levels were higher in severe disease [46.7±24.7 vs. 33.0±12.6 mg/dL, p=0.013].

In contrast, prothrombin time, activated partial thromboplastin time, creatine kinase, total bilirubin, potassium, sodium, and creatinine levels did not differ significantly between the two groups. Laboratory findings of the patients according to COVID-19 disease severity are shown in Table 3.

Cytokine and Chemokine Levels According to Disease Severity

Serum cytokine and chemokine levels were compared between patients with mild and severe COVID-19. Overall, cytokine and chemokine concentrations tended to be numerically higher in patients with severe disease; however, none of the measured mediators showed a statistically significant difference between the groups.

Tumor necrosis factor-alpha levels were higher in the severe disease group than in the mild disease group [48.0±53.9 vs. 30.7±11.6 pg/mL], but the difference was not statistically significant (p=0.644). Interferon-gamma levels were also higher in severe disease [14.3±26.3 vs. 6.5±7.1 pg/mL], without statistical significance (p=0.271).

Interleukin-13 levels were slightly higher in severe disease [1.6±0.7 vs. 1.3±0.3 pg/mL], but again, the difference did not reach statistical significance (p=0.119). Monocyte chemoattractant protein-1 levels were numerically higher in severe disease [159.5±262.8 vs. 86.4±51.7 pg/mL], but this difference was not significant (p=0.533). Macrophage inflammatory protein-1 beta levels were also higher in severe disease [54.1±89.2 vs. 38.6±15.6 pg/mL], with no statistically significant difference between the groups (p=0.854).

These findings indicate that, despite a numerical upward trend in selected cytokine and chemokine levels among patients with severe COVID-19, pretreatment single-timepoint measurements of TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β did not significantly discriminate between mild and severe disease. Admission serum cytokine and chemokine levels according to COVID-19 disease severity are presented in Table 4.

Discussion

In this single-center, cross-sectional study, we evaluated pretreatment admission serum cytokine and chemokine levels in patients with mild and severe COVID-19 and

Table 3. Laboratory findings according to COVID-19 disease severity

Laboratory findings	All (78) Mean±SD	Severe (46) Mean±SD	Mild (32) Mean±SD	p
Whice cell count (x 10^9/ L, 3.7-10.4)	6.8±3.4	7.8±3.7	5.4±2.2	0.002
Neutrophil count (x 10^9/ L, 1.8-7.8)	5.1±3.2	6.1±3.7	3.7±1.9	0.001
Lymphocyte count (x 10^9/ L, 0.9-3.7)	1.2±0.6	1±0.6	1.5±0.6	**<0.001**
Platelet count (x 10^9/ L, 149-371)	240±85.7	216.2±52.6	257.4±100.6	0.045
Protrombin time (s, 9.7-13.9)	13.3±3.7	13.1±3.9	13.8±2.9	0.549
Active partial thromboplastin time (s, 22-45)	33.8±11	33.4±9.5	35.1±14.6	0.616
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
ALT (U/L, 0-41)	47.1±47.9	57.5±55.3	32.4±29.9	0.03
AST (U/L, 0-40)	44.7±42.5	57.3±50.9	26.6±11.5	0.002
CK (IU/L, 0-190)	142.1±125.7	154.7±137.3	108.5±82.1	0.228
LDH (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 level (mmol/L,.5-5.1)	6.1±15.9	7.5±20.8	4.1±0.4	0.384
Sodium level (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**
CRP (mg/dL, 0-0.5)	52.1±84.1	139.2±84.1	1±2.6	**<0.001**

Table 4. Admission serum cytokine and chemokine levels according to COVID-19 disease severity

	Severe (n=46)		Mild (n=32)		
Immune Mediatory	**Mean±SD**	**[Q1:Q3] Median**	**Mean±SD**	**[Q1:Q3] Median**	**p***
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
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

compared their association with disease severity against conventional inflammatory and tissue injury markers. The main finding was that routinely available laboratory parameters, including C-reactive protein, ferritin, lactate dehydrogenase, D-dimer, leukocyte and neutrophil counts, lymphocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, and urea, were significantly associated with severe disease. In contrast, selected cytokines and chemokines, including TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β, were numerically higher in severe COVID-19 but did not reach statistical significance. This pattern suggests that conventional inflammatory and tissue injury markers may better reflect the integrated systemic burden of COVID-19 at admission than isolated single-time-point cytokine measurements.

Patients with severe disease in our cohort were older, more frequently male, and had a higher burden of comorbidities, particularly hypertension, diabetes mellitus, cardiovascular disease, and chronic obstructive pulmonary disease. These findings are consistent with the recognized clinical phenotype of severe COVID-19, in which host vulnerability substantially modifies the inflammatory response to SARS-CoV-2 infection. Age, cardiometabolic disease, and chronic pulmonary disease may amplify endothelial dysfunction, basal inflammatory tone, coagulation activation, and susceptibility to organ injury. Therefore, the stronger association of conventional markers with severe disease may partly reflect their ability to capture both acute infection-related inflammation and the underlying systemic vulnerability of the host.[14,15]

The clearest laboratory differences between mild and severe disease were observed in CRP, ferritin, LDH, D-dimer, neutrophil count, lymphocyte count, and platelet count. This is biologically plausible. CRP is a downstream marker of hepatic acute-phase activation, largely driven by upstream inflammatory signaling, such as the IL-6 pathway. Ferritin reflects macrophage activation, cellular stress, and systemic hyperinflammation. LDH indicates tissue injury and cellular turnover. D-dimer reflects coagulation activation, endothelial injury, and thromboinflammation. Neutrophilia and lymphopenia reflect the combined effects of innate immune activation, stress response, and impaired adaptive immune regulation. Thrombocytopenia may reflect platelet consumption, endothelial activation, inflammation-associated coagulopathy, or severe systemic illness. Systematic reviews of COVID-19 biomarkers have similarly identified D-dimer, ferritin, neutrophil-to-lymphocyte ratio, and related hematologic or inflammatory markers as clinically relevant indicators of disease severity and mortality.[9,10]

Importantly, our panel did not include IL-6 or IL-8, two of the most consistently reported cytokines associated with severe COVID-19. Therefore, our findings should not be interpreted as evidence against cytokine involvement in COVID-19, but rather as evidence that the selected cytokines and chemokines measured at a single admission time point did not outperform conventional markers.

Although all measured cytokines and chemokines were numerically higher in the severe group, large standard deviations suggest substantial interindividual variability. In a modest-sized cohort, such variability may reduce statistical power and obscure biologically meaningful trends. Our analysis reflects disease severity at admission, not subsequent clinical outcomes.

TNF-α may be associated with endothelial activation, inflammation, and tissue damage in severe COVID-19. Del Valle and colleagues demonstrated that elevated levels of IL-6, IL-8, and TNF-α at the time of hospital admission were strong and independent predictors of survival.[7] In our study, TNF-α was also elevated, but not significantly; this may be explained by the sample size and variability. IFN-γ is an indicator of the antiviral Th1 response, but timing is critical in COVID-19. While an early antiviral response may be protective, a late and dysregulated response may increase inflammation. Therefore, severity discrimination may be difficult with a single measurement. MCP-1 is associated with monocyte chemotaxis and is important in terms of pulmonary inflammation and monocyte-macrophage acti-

vation.[16,17] In some studies, MCP-1 has been reported to be elevated in COVID-19. In the study by Pinarlik et al., IFN-α2, TNF-α, MCP-1/CCL2, IL-6, IL-8, IL-18, and IL-33 levels were reported to be higher in patients with COVID-19 than in healthy controls, while increased CRP levels and decreased lymphocyte counts were observed in critical illness.[18] MIP-1β is involved in the macrophage- and T-cell-related chemotactic response. Its numerical elevation in the severe group may be biologically expected, but due to wide variation and the small sample size, it may not have reached statistical significance.

The absence of statistically significant differences in TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β should not be interpreted as evidence against cytokine involvement in COVID-19 pathogenesis. Rather, our findings indicate that the selected cytokines and chemokines measured at a single pretreatment admission time point did not discriminate mild from severe disease as effectively as conventional laboratory markers. Cytokines are dynamic rather than static biomarkers. Their circulating concentrations may vary according to disease phase, timing from symptom onset, viral replication kinetics, compartmentalized pulmonary inflammation, host immune status, assay platform, and prior immune priming. Longitudinal cytokine studies have emphasized that early and late cytokine profiles may differ across disease severity groups, supporting the idea that inflammatory trajectories may be more informative than isolated baseline values.[8]

A second explanation is that cytokines may peak earlier or later than the downstream laboratory markers used in routine practice. CRP, ferritin, LDH, and D-dimer are more stable integrated readouts of systemic inflammation, tissue injury, and thromboinflammatory burden. By contrast, cytokines can be transient, pulsatile, locally compartmentalized, and technically more variable. A patient sampled early in the course of the disease may not yet have developed a measurable systemic cytokine increase, whereas another sampled later may already show downstream acute-phase activation after the cytokine peak has passed. Therefore, conventional markers may outperform cytokines at admission because they summarize the biological consequences of inflammation rather than only a narrow upstream signaling snapshot.

Another important point is that our cytokine panel did not include IL-6 or IL-8, two of the most consistently reported cytokines associated with severe COVID-19. Del Valle et al. showed that high serum IL-6, IL-8, and TNF-α levels at hospitalization were strong predictors of survival and that IL-6 and TNF-α remained independently associated with disease severity and death after adjustment for clinical and laboratory variables.[7,19,20] IL-6 is also central to the acute-phase response and has been repeatedly discussed as a key mediator in COVID-19-associated cytokine storm. Therefore, the absence of IL-6 and IL-8 measurements is a relevant limitation of our panel. In our cohort, the marked CRP elevation in severe disease may indirectly reflect upstream IL-6-related acute-phase activation, but this cannot be confirmed without direct IL-6 measurement.

The measured cytokines and chemokines still have biological relevance. TNF-α contributes to endothelial activation, inflammation, vascular permeability, and tissue injury. IFN-γ reflects antiviral Th1 immune activation, but its clinical meaning depends strongly on timing; early interferon signaling may support viral control, whereas delayed or dysregulated responses may contribute to immunopathology. MCP-1, also known as CCL2, promotes monocyte recruitment and may participate in monocyte-macrophage accumulation within inflamed tissues. MIP-1β, also known as CCL4, is involved in the chemotactic recruitment of immune cells and macrophage-associated inflammation. IL-13 is more closely related to type 2 immune responses, mucus production, tissue remodeling, and fibrotic pathways, and it may not be expected to behave as a dominant acute severity marker in the same way as IL-6 or IL-8.[21-23] Thus, the lack of significant group differences in our study may reflect not only sample size and timing but also the fact that not all cytokines are equally suited for acute severity discrimination.

The numerical elevation of all measured cytokines and chemokines in the severe group is nevertheless noteworthy. TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β were all higher in patients with severe disease, but standard deviations were wide, especially for TNF-α, IFN-γ, MCP-1, and MIP-1β. This suggests substantial interindividual variability. In a modest-sized cohort, such variability can reduce statistical power and obscure biologically meaningful trends. Therefore, our results should be interpreted as showing the limited discriminatory performance of these pretreatment single-time-point measurements in this cohort, rather than the complete absence of inflammatory differences between mild and severe disease.

From a clinical perspective, these findings are relevant because emergency department risk stratification requires rapid, reproducible, inexpensive, and easily interpretable biomarkers. Conventional markers, such as CRP, ferritin, LDH, D-dimer, complete blood count parameters, and routine biochemical tests, are widely available and reflect multiple downstream processes, including acute-phase activation, macrophage response, endothelial injury, coagulation activation, immune cell redistribution, and tissue damage. Cytokine profiling, although biologically attractive, is more

expensive, technically demanding, less standardized across platforms, and more sensitive to sampling-time effects. Accordingly, our findings support the view that selected single-time-point cytokine measurements should not be used as standalone admission severity markers but may be more informative when combined with serial sampling, broader cytokine panels, and clinical variables.[24-26]

This study has several strengths. First, cytokine and chemokine measurements were performed using pretreatment blood samples obtained at emergency department admission, reducing the potential confounding effects of corticosteroids, antiviral agents, anticoagulants, or other COVID-19-specific therapies on inflammatory mediator levels. Second, patients were classified according to World Health Organization disease severity criteria, providing a clinically recognizable severity framework. Third, the study directly compared specialized cytokine and chemokine measurements with routinely available laboratory markers, which is relevant for real-world clinical decision-making.

The study also has limitations. It was a single-center study with a relatively small sample size. Cytokines and chemokines were measured at only one time point, preventing the assessment of inflammatory trajectories. The panel did not include IL-6 or IL-8, two cytokines frequently linked to COVID-19 severity. Detailed data on intensive care unit admission, mortality, mechanical ventilation, and oxygen requirement were not available; therefore, the analysis was limited to disease severity at admission rather than subsequent clinical outcomes. In addition, age, sex, and comorbidity differences between severity groups may have influenced both conventional laboratory markers and cytokine levels. Larger studies with serial sampling, broader cytokine panels, and multivariable adjustment are needed to clarify whether cytokine profiling adds independent value beyond conventional markers.

Conclusion

In conclusion, conventional inflammatory, hematological, coagulation-related, and tissue injury markers were more strongly associated with COVID-19 severity at admission than selected pretreatment serum cytokine and chemokine levels. Although TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β were numerically higher in severe disease, none significantly distinguished severe from mild COVID-19. These findings suggest that the apparent superiority of conventional markers may derive from their greater biological stability, clinical accessibility, and ability to reflect integrated systemic inflammation and tissue injury. Cytokine profiling may still be valuable in COVID-19 research, but its clinical utility likely depends on broader inflammatory panels, longitudinal sampling, and integration with routine laboratory and clinical parameters.

Disclosures

Ethics Committee Approval: This study was approved by The Istanbul Faculty of Medicine Clinical Research Ethic Committee (Approval Number: 671545 - 31.12.2021).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

Conflict of Interest: The author declare that there is no conflict of interest.

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