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Cytokines and their relationship with the severity and prognosis of coronavirus disease 2019 (COVID-19): a retrospective cohort study

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ABSTRACT

Objective To delineate the characteristics and clinical significance of plasma inflammatory cytokines altered in COVID-19.

Design Retrospective, single-centre cohort study.

Setting Tongji Hospital in Wuhan, China.

Participants Among a cohort of 308 patients with a diagnosis of COVID-19, 138 patients died while 170 patients recovered and were discharged from the hospital. The data were collected until 27 February 2020.

Primary and secondary outcome measures Clinical characteristics and laboratory findings were obtained from electronic medical records using data collection forms.

Results The percentage of patients with elevated interleukin 2 receptor (IL-2R), IL-6, IL-8, IL-10 and tumour necrosis factor (TNF) increased with severity of disease (p<0.0001 for all). IL-2R (p<0.0001), IL-6 (p<0.0001), IL-8 (p=0.0001), IL-10 (p<0.0001) and TNF (p<0.0001) were also twofold to 20-fold higher in patients who died compared with those who recovered. Also, IL-6 and IL-10 increased in both the progressive patient groups: moderate (p=0.0026) and severe (p<0.0001). In multivariate analysis, higher levels of IL-2R (OR 1.001, 95% CI 1.000 to 1.002, p=0.031) and IL-6 (OR 1.013, 95% CI 1.003 to 1.024, p=0.015) on admission were associated with increasing odds of in-hospital death, independent of other covariates, including severity of disease and lymphocyte count.

Conclusion Increased proinflammatory and anti-inflammatory cytokines, including IL-2R, IL-6, IL-8, TNF and IL-10, showed an obvious association with both COVID-19 severity and in-hospital mortality. Thus, our study indicates that cytokines are valuable in predicting the severity of COVID-19 and helps in distinguishing critically ill patients from the less affected ones.

Strengths and limitations of this study

- This study systematically investigated the cytokine profiles among patients with COVID-19.
- The major finding is that cytokine level is valuable in predicting the severity and prognosis of COVID-19.
- The quality of the study is reduced by lack of observation of the relationship between dynamic cytokine levels and progression of COVID-19.
- This study is a retrospective, single-centre cohort analysis based on initial inflammatory cytokine levels on admission.
- Data regarding lymphocyte subsets are not available.

INTRODUCTION

The COVID-19 outbreak, caused by SARS-CoV-2, is erupting worldwide.[1 2] The WHO declared COVID-19 as a pandemic.[3] Although most cases were mild to moderate, increasing COVID-19 cases led to a significant number of patients developing severe symptoms and death. A lot of detailed clinical information on COVID-19 is currently known, but the characteristics of inflammatory cytokines associated with patients with COVID-19 remain largely unclear.

Severe illness, of almost any aetiology, is accompanied by a generalised host inflammatory response. This host immune response process is referred to as systemic inflammatory response syndrome. If this process is not controlled or is dysfunctional, it will lead to cytokine storm syndrome.[4] Cytokine storm is one of the possible mechanisms underlying rapid disease progression.[5] Recent studies have reported a relationship between serum inflammatory cytokine levels and severity or prognosis of patients with COVID-19. However, there are some inconsistent conclusions. For example, several studies reported interleukin (IL)-6 as a potential biomarker for predicting COVID-19 progression or monitoring disease severity.[6–8] However, Song *et al*[9] found no significant differences in the level of IL-6 and tumour necrosis factor (TNF) between severe and non-severe patients with COVID-19. Most of the previous studies only focused on the role of IL-6 in COVID-19. The characteristics and the role of other cytokines in COVID-19, especially the anti-inflammatory

cytokine IL-10, have received little attention. IL-10 can be a parameter in predicting the clinical outcome of patients with severe community-acquired pneumonia and is also the most important anti-inflammatory cytokine in human immune response.[10]

Thus, the authors of this research have systematically studied cytokine profiles along with their relationship with the severity and prognosis of COVID-19. Meanwhile, objective factors of patients related to their basic level of cytokines were explored. Earlier identification of a severe or a critical case can help in providing clinical intervention on time.

METHODS

Study design and participants

This was a retrospective, single-centre study which enrolled 308 patients from 10 January 2020 to 13 February 2020 at Tongji Hospital, Huazhong University of Science and Technology (Wuhan, China). As of 27 February 2020, 138 patients have died while 170 have recovered and were discharged from the hospital. Data were collected at the time of admission. Diagnosis and clinical classification of COVID-19 were made according to the clinical guidelines (version 5 trial) developed by the National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/). The clinical classification is as follows:

- Moderate type: including fever, respiratory tract symptoms and imaging that shows pneumonia.
- Severe type: meeting any of the following criteria: (1) respiratory distress, respiratory rate ≥30 beats/min; (2) in resting state, meaning oxygen saturation ≤93%; and (3) arterial blood oxygen partial pressure/oxygen concentration ≤300 mm Hg (1 mm Hg=0.133 kPa).
- Critical type: involving one of the following conditions: (1) respiratory failure requiring mechanical ventilation; (2) presence of shock; and (3) combined organ failure requiring admission in intensive care unit.

Tongji Hospital is a designated hospital for critically ill patients. In this cohort, we observed relatively high mortality. Exacerbation from moderate to severe, or severe to critical, was defined as progression. The primary outcome of the study was in-hospital mortality. The study excluded patients with secondary vasculitis and uraemia that needed maintenance on haemodialysis, along with the ones who died within 48 hours of admission. Before enrolment, written informed consent was obtained from patients involved in the study, while data were collected retrospectively.

Cytokine measurement

In order to explore the influence of COVID-19 on the secretion of cytokines, chemiluminescence immunoassay (CLIA) was performed. Cytokines including IL-1β, interleukin 2 receptor (IL-2R), IL-6, IL-8 (also known as CXCL8), IL-10 and TNF were assessed using serum samples drawn from patients shortly after hospitalisation. CLIA was performed using a fully automated analyser (Immulite 1000, DiaSorin Liaison, Italy; or Cobas e602, Roche Diagnostics, Germany) for all the patients according to the manufacturer's instructions. IL-1β kit (#LKL11), IL-2R kit (#LKIP1), IL-8 kit (#LK8P1), IL-10 kit (#LKXP1) and TNF kit (#LKNF1) were purchased from DiaSorin (Vercelli, Italy). IL-6 kit (#05109442190) was purchased from Roche Diagnostics. The patients were divided into elevated and normal groups according to the instructions.

Statistical analysis

Continuous variables were expressed as a median with IQR, and categorical variables were described as frequency rates and percentages. We used Mann-Whitney U test or Kruskal-Wallis test, whichever was appropriate, to compare differences between groups. Proportions for categorical variables were compared using χ^2 test, Fisher's exact test or Yates' continuity corrected χ^2 test. Univariate and multivariate logistic regression models (forward: likelihood ratio (LR)) were applied to screen the risk of death. The Kaplan-Meier method was used to assess the cumulative rate of mortality based on the normal range of cytokines and was compared with the log-rank test. Receiver operating characteristic curve (ROC) analysis was performed to assess the accuracy of inflammatory cytokine levels in predicting death. The tests were two-sided, and a p value less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS V.23.0.

Patient and public involvement

This was a retrospective, single-centre cohort study and no patients were involved in the study design or in setting the research questions or the outcome measures directly. No patients were asked for advice on interpretation or writing of the results.

RESULTS

Characteristics of COVID-19 patient cohort

The demographic and clinical characteristics of 308 patients with COVID-19 are summarised in table 1. At the time of admission, 91 (30%) patients were classified as moderate type, 133 (43%) patients as severe, and 84 (27%) patients fulfilled the criteria of critical type. As per the results, 138 (45%) patients died and 170 (55%) were discharged from the hospital. Moderately ill and surviving patients were more likely to be female and younger. The ratio of comorbidity was significantly higher in critical (p<0.0001) and deceased (p<0.0001) group. In the laboratory findings, the levels of lymphocytes, platelets and total cholesterol were lower in the critical and deceased groups, whereas neutrophil count, D-dimer, alanine aminotransferase, blood urea nitrogen, creatinine, procalcitonin, lactic dehydrogenase, C reactive protein (CRP), IL-2R, IL-6, IL-8, IL-10 and TNF were significantly higher (p<0.0001 for all). Also, the critical

Table 1 Demographics and clinical characteristics of patients with COVID-19

Variables	Condition on admission				Outcome		
	Moderate (n=91)	Severe (n=133)	Critical (n=84)	P value	Survivor (n=170)	Non-survivor (n=138)	P value
Age, years	55.0 (39.0–67.0)	66.0 (55.5–73.0)	70.0 (63.3–78.8)	<0.0001	57.0 (43.0–68.0)	70.0 (62.8–78.0)	<0.0001
Female, n (%)	51/91 (56)	67/133 (50)	24/84 (29)	0.0003	95/170 (56)	47/138 (34)	0.0001
Comorbidity, n (%)	35/89 (39)	82/132 (62)	61/83 (74)	<0.0001	72/167 (43)	106/137 (77)	<0.0001
Hypertension	18/89 (20)	51/132 (39)	41/83 (49)	<0.0001	43/167 (26)	67/137 (49)	<0.0001
Diabetes	14/89 (16)	22/132 (17)	20/83 (24)	0.1621	24/167 (14)	32/137 (23)	0.0443
Cardiovascular disease	5/89 (6)	21/132 (16)	15/83 (18)	0.0158	13/167 (8)	28/137 (20)	0.0013
Cerebrovascular disease	2/89 (2)	3/132 (2)	7/83 (8)	0.0402	4/167 (2)	8/137 (6)	0.2155
Pulmonary disease	6/89 (7)	11/132 (8)	9/83 (11)	0.3380	10/167 (6)	16/137 (12)	0.0775
Chronic kidney disease	0/89 (0)	3/132 (2)	4/83 (5)	0.0353	1/167 (1)	6/137 (4)	0.0715
Signs and symptoms, n (%)							
Fever	76/90 (84)	119/133 (90)	77/84 (92)	0.1318	145/169 (86)	127/138 (92)	0.0875
Cough	54/90 (60)	101/132 (77)	71/84 (85)	0.0002	114/169 (68)	112/137 (82)	0.0047
Laboratory parameters							
Neutrophil count, $\times 10^9$/L	3.1 (2.5–4.1)	4.0 (2.7–5.7)	10.1 (7.2–14.7)	<0.0001	3.3 (2.4–4.3)	8.1 (4.9–12.5)	<0.0001
Lymphocyte count, $\times 10^9$/L	1.2 (0.9–1.6)	0.9 (0.7–1.3)	0.5 (0.4–0.8)	<0.0001	1.1 (0.8–1.5)	0.6 (0.4–0.8)	<0.0001
Platelet count, $\times 10^9$/L	213.0 (160.0–309.0)	208.5 (149.0–261.3)	156.0 (94.5–222.0)	<0.0001	220.0 (170.8–291.3)	151.0 (107.0–222.5)	<0.0001
D-dimer, µg/mL	0.4 (0.3–0.8)	1.0 (0.5–2.6)	18.1 (2.6–21.0)	<0.0001	0.5 (0.3–1.0)	7.9 (1.3–21.0)	<0.0001
ALT, U/L	18.0 (13.0–24.0)	23.0 (16.0–39.0)	33.5 (21.0–55.8)	<0.0001	20.0 (13.8–32.5)	28.0 (18.0–46.5)	<0.0001
BUN, mmol/L	3.7 (3.1–4.7)	5.1 (3.7–6.7)	9.6 (7.0–16.1)	<0.0001	3.9 (3.1–5.2)	8.8 (5.6–12.9)	<0.0001
Creatinine, µmol/L	66.0 (57.0–81.0)	72.0 (56.0–93.0)	87.5 (70.5–120.0)	<0.0001	65.5 (57.0–82.0)	86.5 (66.8–120.0)	<0.0001
Total cholesterol, mmol/L	3.8 (3.2–4.5)	3.5 (3.1–3.9)	3.2 (2.8–3.8)	<0.0001	3.6 (3.2–4.3)	3.3 (2.8–3.9)	<0.0001
Procalcitonin, ng/mL	0.05 (0.04–0.09)	0.09 (0.05–0.23)	0.49 (0.17–1.49)	<0.0001	0.05 (0.04–0.09)	0.31 (0.14–1.09)	<0.0001
Lactic dehydrogenase, U/L	229.0 (190.0–283.0)	315.0 (219.5–431.8)	637.0 (490.5–872.5)	<0.0001	243.0 (194.8–309.8)	524.5 (366.0–721.0)	<0.0001
C reactive protein, mg/L	7.7 (2.5–24.3)	49.3 (8.9–93.3)	112.3 (71.4–187.3)	<0.0001	10.7 (2.4–36.7)	100.5 (62.4–161.2)	<0.0001
Interleukin 1β ≥5 pg/mL, n (%)	13/91 (14)	17/133 (13)	11/84 (13)	0.8120	23/170 (14)	18/138 (13)	0.9006
Interleukin 2 receptor, U/mL	475.5 (375.8–630.8)	799.0 (538.5–1097.0)	1259.5 (942.3–1825.0)	<0.0001	553.0 (402.0–802.0)	1137.5 (822.0–1584.3)	<0.0001
≥710 U/L, n (%)	19/91 (21)	74/133 (56)	77/84 (92)	<0.0001	51/170 (30)	113/138 (82)	<0.0001
Interleukin 6, pg/mL	5.6 (2.7–15.3)	24.3 (6.7–61.7)	64.8 (29.42–153.1)	<0.0001	7.9 (2.7–22.8)	59.7 (23.6–137.4)	<0.0001
≥7 pg/mL, n (%)	41/91 (45)	100/133 (75)	77/83 (92)	<0.0001	90/170 (53)	132/137 (96)	<0.0001
Interleukin 8, pg/mL	15.4 (7.7–29.4)	19.5 (12–35.5)	30.8 (21.0–71.8)	<0.0001	16.3 (9.4–28.7)	26.6 (16.4–60.40)	<0.0001
≥62 pg/mL, n (%)	10/91 (11)	13/133 (10)	77/84 (92)	<0.0001	14/170 (8)	33/138 (24)	0.0001

Continued

Table 1 Continued

Variables	Condition on admission				Outcome		
	Moderate (n=91)	Severe (n=133)	Critical (n=84)	P value	Survivor (n=170)	Non-survivor (n=138)	P value
Interleukin 10, pg/mL	5.0 (5.0–5.1)	5.9 (5.0–10.80)	10.9 (6.4–18.7)	<0.0001	5.0 (5.0–6.7)	10.1 (5.4–16.4)	<0.0001
≥9.1 pg/mL, n (%)	10/91 (11)	46/133 (35)	76/83 (92)	<0.0001	31/170 (18)	76/137 (56)	<0.0001
TNF, pg/mL	7.7 (6.0–9.5)	8.6 (6.9–11.9)	11.2 (7.4–18.8)	<0.0001	7.8 (6.1–9.7)	10.9 (7.7–15.9)	<0.0001
≥8.1 pg/mL, n (%)	43/91 (47)	80/133 (60)	77/84 (92)	<0.0001	82/170 (48)	101/138 (73)	<0.0001
Treatment, n (%)							
Mechanical ventilation	4/91 (4)	46/133 (35)	79/84 (94)	<0.0001	7/170 (4)	122/138 (88)	<0.0001
Antibiotics treatment	85/91 (93)	128/133 (96)	84–0/84 (100)	0.0191	160/170 (94)	137/138 (99)	0.0343
Antiviral treatment	90/90 (100)	124/126 (98)	62/79 (79)	<0.0001	167/167 (100)	109/128 (85)	<0.0001
Corticosteroids	48/90 (53)	86/133 (65)	74/84 (88)	<0.0001	84/169 (50)	124/138 (90)	<0.0001
Immunoglobulin	31/91 (34)	77–56/133 (58)	47–37/84 (56)	0.0031	69/170 (41)	86/138 (62)	0.0001
Duration of complaint, days	8.0 (4.8–13.0)	10.0 (7.0–13.5)	11.0 (7.0–15.0)	0.0007	8.0 (6.0–13.0)	10.0 (7.0–15.0)	0.0027
Hospitalisation, days	16.0 (12.0–23.0)	19.0 (12.0–24.0)	8.0 (4.0–13.0)	<0.0001	19.5 (14.0–24.0)	10.0 (5.0–17.0)	<0.0001
Duration of disease, days	26.0 (20.0–31.0)	29.0 (24.0–35.0)	19.0 (15.0–29.0)	<0.0001	29.0 (24.0–34.0)	22.0 (15.8–30.0)	<0.0001
Progression, n (%)	8/45 (18)	52/129 (40)	–	–	–	–	–

Data are median (IQR), mean (SD) or n (%).
P values were calculated by Mann-Whitney U test, Kruskal-Wallis test, χ^2 test, Fisher's exact test or Yates' continuity corrected χ^2 test, as appropriate.
Duration of complaint: time from onset of symptom to hospital admission; duration of disease: time from onset of symptom to outcome; hospitalisation: time from hospital admission to outcome.
ALT, alanine aminotransferase; BUN, blood urea nitrogen; TNF, tumour necrosis factor.

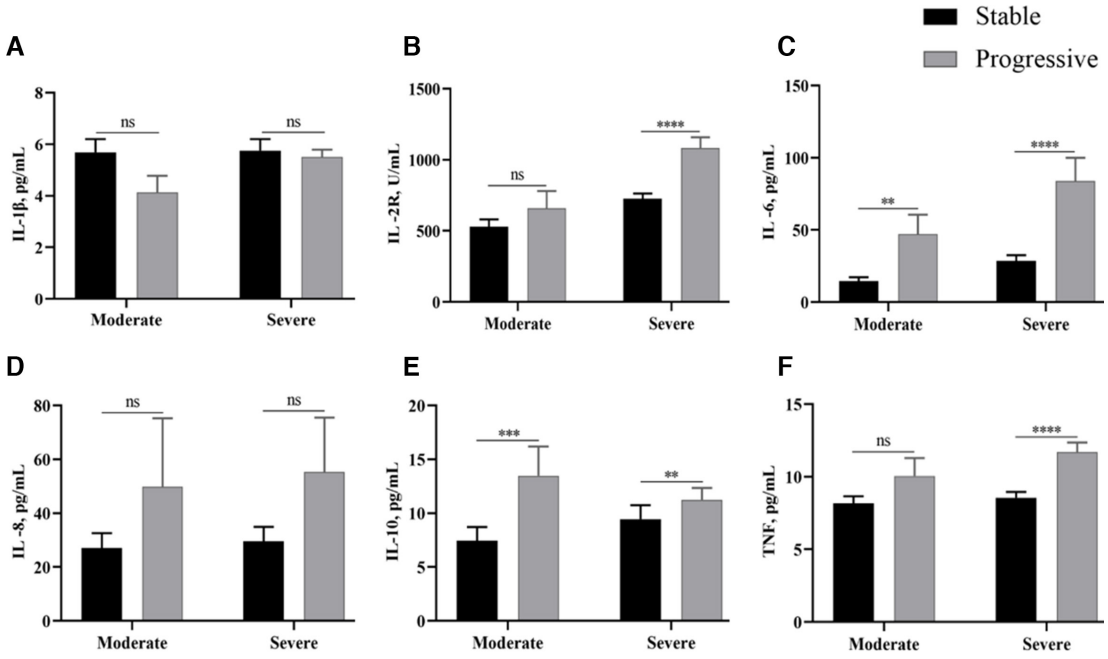

Figure 1 Cytokine values for patients with COVID-19 on admission versus the progressive and stable groups in both moderate and severe types. The error bars represent mean±SEM. P values were calculated by Mann-Whitney U test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. IL, interleukin; IL-2R, interleukin 2 receptor; TNF, tumour necrosis factor. (A) IL-1β, (B) IL-2R, (C) IL-6, (D) IL-8, (E) IL-10, and (F) TNF.

(p<0.0001) and deceased (p<0.0001) groups were more likely to receive non-invasive ventilation or invasive ventilation. The median time from disease onset to outcome was 22 (IQR 15.8–30.0) days for non-survivors and 29 (IQR 24.0–34.0) days for survivors.

Plasma cytokine alteration in COVID-19

The baseline for cytokine concentrations was determined from blood obtained at the time of admission. The levels and the abnormal ratio of cytokines, including IL-2R, IL-6, IL-8, IL-10 and TNF, gradually increased as the disease progressed or resulted in poor prognosis (p<0.0001 for all). No significant difference was observed in IL-1β (p=0.8120) within each of the groups. Likewise, IL-2R (p<0.0001), IL-6 (p<0.0001), IL-8 (p=0.0001), IL-10 (p<0.0001) and TNF (p<0.0001) were also twofold to 20-fold higher in patients who died compared with those who recovered (table 1). Additionally, several cytokines, including proinflammatory and anti-inflammatory, were elevated at baseline in patients whose conditions were progressive compared with patients whose conditions were stable (figure 1). Specifically, the proinflammatory IL-2R (p<0.0001) and TNF (p<0.0001) were significantly higher in the progressive group compared with the stable group in patients of severe type. IL-6 was increased in the progressive group, both in moderate (p=0.0026) and in severe (p<0.0001) types of patients. Although numerically high, the differences in IL-8 levels between the two groups did not reach statistical significance. Similarly, the progressive group had higher levels of the anti-inflammatory cytokine IL-10 in both types of severity (p=0.0008 for moderate type, p=0.0011 for severe type). These results suggest that the progression of COVID-19 was associated with the initial levels of plasma cytokine.

Correlation between baseline cytokine levels and physiological variables measured on admission

The relationship between plasma cytokines and various physiological variables was assessed and is presented in table 2. Except for IL-1β, other plasma cytokines were positively correlated with age, albumin, creatinine, random blood glucose, D-dimer, lactic dehydrogenase and CRP (p<0.001 for all). Also, IL-2R (p=0.002), IL-6 (p<0.001) and IL-10 (p<0.001) were negatively correlated with total cholesterol. There was no significant relationship between the levels of cytokine and of C3 and C4.

Association of plasma cytokines with in-hospital death

Kaplan-Meier analysis indicated a significantly higher mortality rate in patients with abnormal plasma cytokine values, including elevated IL-2R, IL-6, IL-8, IL-10 and TNF (p<0.0001 for all) (figure 2). Univariate logistic regression analysis revealed that IL-2R, IL-6, IL-8, IL-10 and TNF were related to a poor outcome (table 3). After adjusting for age, gender, comorbidities, disease severity and lymphocyte count, the levels of IL-2R and IL-6 were associated with in-hospital mortality (figure 3). Further, plasma cytokines were analysed by ROC analysis to evaluate their ability to predict in-hospital death rates (figure 4). The area under the curve was 0.82 (95% CI 0.78 to 0.87) for IL-2R, 0.85 (95% CI 0.81 to 0.89) for IL-6, 0.69 (95% CI 0.64 to 0.75) for IL-8, 0.75 (95% CI 0.69 to 0.81) for IL-10, and 0.71 (95% CI 0.65 to 0.77) for TNF (table 4).

Table 2 Correlation among baseline inflammatory biomarkers and physiological variables measured on the day of admission

	IL-1β	IL-2R	IL-6	IL-8	IL-10	TNF
Age	−0.002	0.426	0.356	0.293	0.261	0.361
	0.967	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Time from onset of symptom to hospital admission	−0.061	0.222	−0.011	0.038	−0.069	−0.055
	0.289	<0.001*	0.843	0.504	0.226	0.336
Albumin	0.177	−0.527	−0.467	−0.319	−0.346	−0.290
	0.003*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
ALT	−0.047	0.276	0.250	0.198	0.167	0.248
	0.415	<0.001*	<0.001*	0.001*	0.003*	<0.001*
Creatinine	−0.037	0.332	0.355	0.226	0.284	0.439
	0.512	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Uric acid	0.033	0.170	0.155	0.128	0.052	0.331
	0.578	0.004*	0.054	0.031*	0.387	<0.001*
Total cholesterol	−0.021	−0.177	−0.332	−0.080	−0.276	−0.103
	0.709	0.002*	<0.001*	0.159	<0.001*	0.071
Random blood glucose	0.004	0.303	0.276	0.272	0.336	0.270
	0.950	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
D-dimer	−0.026	0.614	0.509	0.328	0.378	0.367
	0.651	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Lactic dehydrogenase	−0.065	0.598	0.562	0.339	0.472	0.391
	0.255	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
CRP	0.008	0.618	0.776	0.345	0.528	0.493
	0.894	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
C3	−0.226	−0.235	−0.026	−0.066	0.093	−0.262
	0.52	0.137	0.882	0.652	0.576	0.075
C4	0.62	0.001	−0.056	−0.156	0.070	−0.21
	0.053	0.991	0.711	0.295	0.673	0.163

Values represent Spearman's correlation coefficients (upper) and p values (lower).
*Denotes statistically significant correlations.
ALT, alanine aminotransferase; CRP, C reactive protein; IL, interleukin; IL-2R, interleukin 2 receptor; TNF, tumour necrosis factor.

DISCUSSION

COVID-19 has rapidly spread throughout the world and has been labelled a pandemic by WHO. Both clinical features and serum markers associated with the severity of patients with COVID-19 have been reported.[11–14] However, we do not know the exact reasons for the specific alterations in cytokine levels; it might be due to the immune response triggering the rapid disease progression. In the present study, we systematically analysed immunological characteristics, particularly cytokine profiles, and their relationship with severity, mortality and prognosis of COVID-19.

Consistent with a previous report, we noted lymphocytopaenia and increased inflammatory cytokine concentration in the majority of severe and critical cases, which were markedly worse when compared with the moderate cases. This indicates that an impaired immune system and a cytokine storm might be associated with COVID-19 severity. Further, the plasma concentration of cytokines in the progressive group, was higher than the stable group in severe cases, although differences in IL-2R, IL-8 and TNF between the two groups did not reach statistical significance in moderate cases, which might be partly due to the limited sample size; a recent study has shown that elevated cytokines were dynamically correlated with disease severity.[5 15 16] Additionally, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) infections were also characterised by elevated levels of inflammatory cytokines with severe lung injury.[17–20]

Cytokines are proteins, glycoproteins or signalling peptides with potent various biological functions at picomolar concentration.[21] IL-2R, IL-6, IL-8 and TNF were major proinflammatory factors required to initiate a series of effective immune cascade events to an infection or a tissue injury site. The anti-inflammatory IL-10 was found to inhibit the monocyte inflammatory response directly and negatively regulate the cascade of proinflammatory cytokines, which induced monocyte hyporesponsiveness in sepsis and multiple organ dysfunction.[22] It is reported

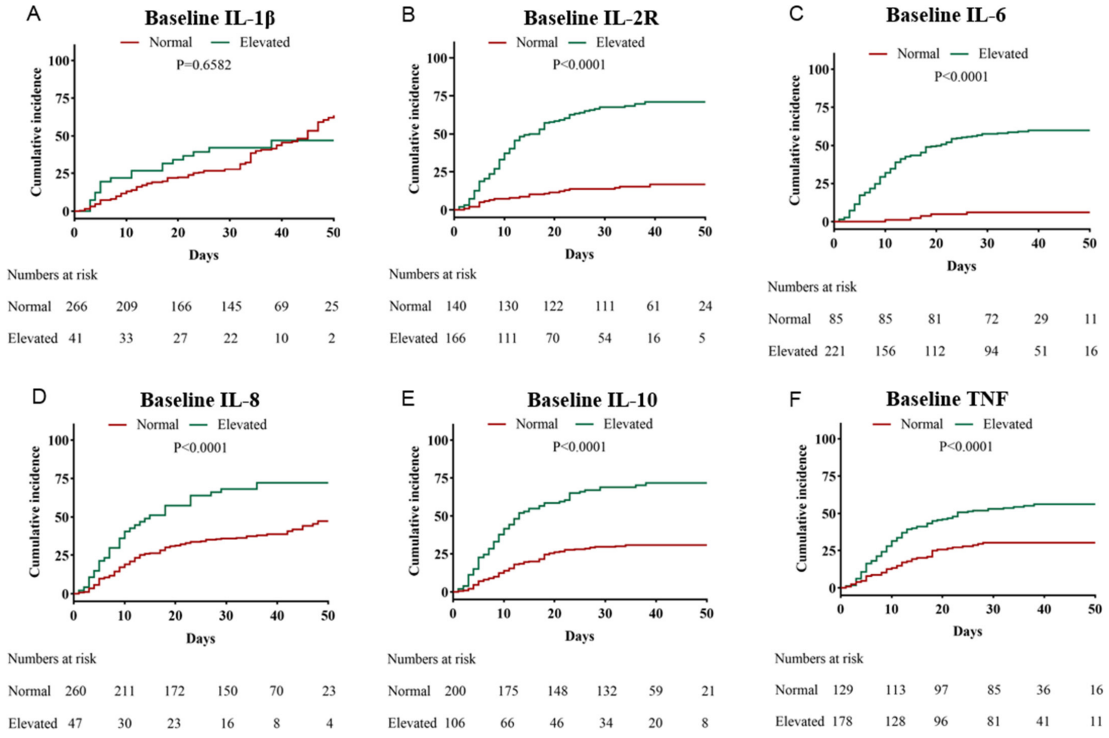

Figure 2 Cumulative incidence of in-hospital mortality in patients with COVID-19 subgrouped by cytokines: (A) IL-1β, (B) IL-2R, (C) IL-6, (D) IL-8, (E) IL-10 and (F) TNF. IL, interleukin; IL-2R, interleukin 2 receptor; TNF, tumour necrosis factor.

that coronavirus infection, its rapid replication, as well as the delayed type I interferon (IFN-I)signalling activate inflammatory monocyte-macrophage, resulting in an increased cytokine concentration, vascular leakage and pathogenic T cell response.[23] Besides, lymphocytopaenia observed in severe and critical patients may impair T cells, which dampen the overactive innate immune response and further aggravate the inflammatory response.[24] In line with the previous study, we suggest that excessive cytokine secretion may be associated with the progressive group. Nevertheless, the underlying cellular source and the mechanism involving cytokine accumulation remain to be determined.

Meanwhile, at the time of admission, serum levels of both proinflammatory and anti-inflammatory cytokines, including IL-2R, IL-6, IL-8, TNF and IL-10, were significantly higher in the case of non-survivors compared with that of the survivors. Previous studies have shown that proinflammatory cytokines predict mortality in patients with sepsis, acute respiratory distress syndrome and the severe infection seen after burn injuries.[20 25] Also, elevated cytokines are a concern as an independent risk factor for poor outcome in acute renal failure.[26] In line

Table 3 Logistic regression analysis of independent factors for predicting mortality of patients with COVID-19

Cytokines	P value	OR	95% CI
IL-1β, pg/mL*	0.242	1.028	0.982 to 1.077
IL-2R, U/mL*	<0.001	1.003	1.002 to 1.004
IL-6, pg/mL*	<0.001	1.031	1.022 to 1.040
IL-8, pg/mL*	<0.001	1.010	1.004 to 1.015
IL-10, pg/mL*	<0.001	1.066	1.032 to 1.101
TNF, pg/mL*	<0.001	1.205	1.131 to 1.285

*Per one-unit increase.
IL, interleukin; IL-2R, interleukin 2 receptor; TNF, tumour necrosis factor.;

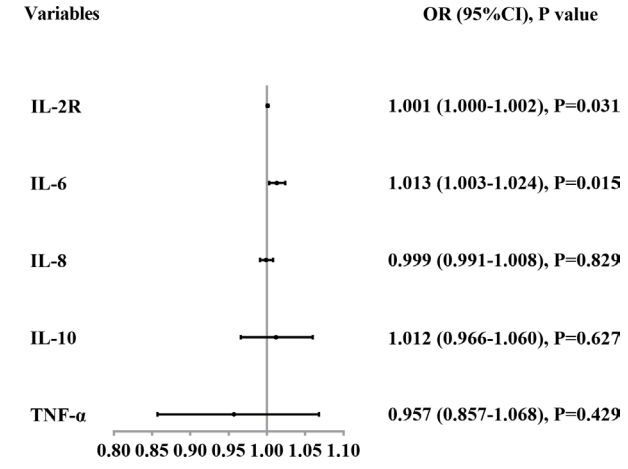

Figure 3 Association of inflammatory cytokines with in-hospital mortality in patients with COVID-19. ORs of each variable were obtained using multivariate logistic regression models after adjustment for age, gender, comorbidities, disease severity and lymphocyte count. Severity was staged based on the guidelines for diagnosis and treatment of COVID-19 (trial fifth edition) published by the Chinese National Health Commission on 4 February 2020. IL, interleukin; IL-2R, interleukin 2 receptor; TNF, tumour necrosis factor.

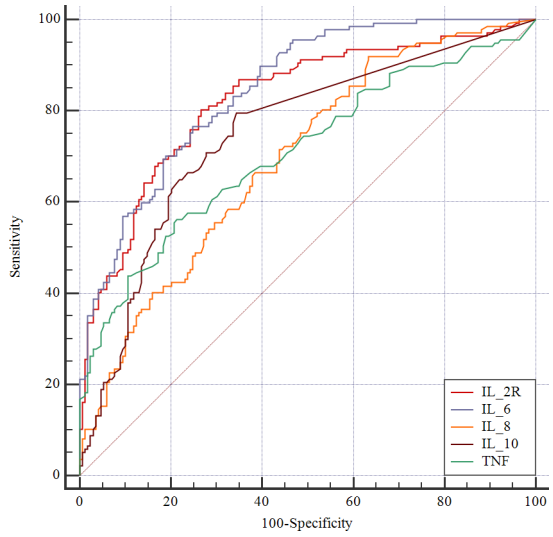

Figure 4 Receiver operating characteristic curve of IL-2R, IL-6, IL-8, IL-10 and TNF for in-hospital death. IL, interleukin; IL-2R, interleukin 2 receptor; TNF, tumour necrosis factor.

with previous research,[27] univariate logistic regression analysis suggested that IL-2R, IL-6, IL-8, IL-10 and TNF were related to in-hospital mortality incidents in the present cohort. The association between IL-2R, IL-6 and in-hospital mortality was maintained after adjusting for demographics and other confounders. A similar conclusion of elevated cytokines related to in-hospital mortality has been found in SARS-CoV or MERS-CoV infection.[18–20] IL-1 is released after the binding of SARS-CoV-2 to the Toll-like receptor (TLR), then mediates lung inflammation, fever and fibrosis, and provokes severe respiratory problems.[28 29] However, both concentration and proportion of IL-1β, in contrast, were not increased in most of the patients, with only 13% of non-survivors showing elevated levels of IL-1β. Meanwhile, the secondary inflammatory cytokine IL-6, considered more distal than IL-1 in the inflammatory cascade, was a significant predictor of survival and had a higher area under the curve value. The specific immune cascade response and the cellular origin of cytokines in COVID-19 deserve further exploration.

At present, many drugs with variable efficacies have been proposed for the treatment of COVID-19-induced cytokine storm.[28–31] The interplay of the main proinflammatory IL-6 and TNF contributes to the cytokine storm. Thus, the targeted therapy of IL-6 and TNF should not be neglected in patients with COVID-19. Tocilizumab, a specific monoclonal antibody that blocks IL-6, has been used for patients with severe COVID-19 with confirmed elevated levels of IL-6.[30] Previous reports have shown that the use of anti-IL-6 treatment with tocilizumab led to a reduction in fever and lung lesion opacity, and recovered the percentage of lymphocytes in peripheral blood.[32] Chloroquine and hydroxychloroquine can suppress the production of various cytokines, such as IL-1, IL-6 and TNF, via TLR signalling and cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS) stimulation of interferon genes.[33] However, the therapeutic benefit of chloroquine in patients with COVID-19 remains controversial.[34 35] Anti-TNF therapy is used in some patients with COVID-19 with autoimmune diseases. A case report showed that treatment with anti-TNF seems to have a protective effect on the evolution of severe types, thereby preventing the damaging effects of cytokine storm.[36] However, anti-TNF has been associated with an increased risk of respiratory complications or death.[37] Overall, immunomodulatory agents with good safety profiles for severe COVID-19 remain limited.

Our study had some limitations. First, this study is a retrospective analysis based on the initial inflammatory cytokines on admission. We did not describe the kinetic change in cytokine profiles of patients with COVID-19. The function of cytokine and the role of cytokine accumulation for pulmonary and other organs remain to be elucidated. Therefore, the relationship between prognosis significance and time-dependent changes in cytokines remains unknown. Second, since data regarding the lymphocyte subsets are not available, further studies are needed to analyse the correlation between the change in lymphocyte subsets and humoral immune response. Third, Tongji Hospital was a designated hospital for severely or critically ill patients with COVID-19, so there was bias in critical patient selection for prognostic research. Thus, the case fatality ratio in our study cannot reflect the true mortality of COVID-19. Last but not least, this was a retrospective, observational, single-centre study. Whether the results of the present study are applicable to other regions is questionable due to the potential differences in treatment protocol and time for patients to receive treatment.

Table 4 Analysis of receiver operating characteristics curve for predicting in-hospital mortality of patients with COVID-19

Variables	P value	AUC (95% CI)	Sensitivity	Specificity	Cut-off value	PPV	NPV
IL-2R	<0.001	0.82 (0.78 to 0.87)	0.80	0.73	755.50	0.72	0.55
IL-6	<0.001	0.85 (0.81 to 0.89)	0.77	0.75	22.80	0.71	0.80
IL-8	<0.001	0.69 (0.64 to 0.75)	0.66	0.62	20.50	0.58	0.55
IL-10	<0.001	0.75 (0.69 to 0.81)	0.80	0.66	5.15	0.65	0.55
TNF	<0.001	0.71 (0.65 to 0.77)	0.56	0.79	10.05	0.68	0.55

AUC, area under curve; IL, interleukin; IL-2R, interleukin 2 receptor; NPV, negative predictive value; PPV, positive predictive value; TNF, tumour necrosis factor.

In conclusion, both proinflammatory and anti-inflammatory cytokine alterations, including IL-2R, IL-6, IL-8, TNF and IL-10, show an obvious association with severity and in-hospital mortality in patients with COVID-19.

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