Dynamic changes in serum IL-6, IL-8, and IL-10 are associated with the outcome of patients with severe COVID-19 in ICU

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Research Article

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Abstract

Background: Biomarkers that would help prognosticate outcomes and guide treatment of patients with severe coronavirus disease 2019 (COVID-19) are currently required. We aimed to investigate whether the dynamic variation of cytokines was associated with the survival of patients admitted to an intensive care unit (ICU).

Methods: A retrospective study was performed on 40 patients with COVID-19 admitted to an ICU in Wuhan, China. Demographic, clinical, and laboratory variables were collected, and serum cytokines were kinetically assessed. A multivariable- adjusted generalized linear regression model was used to evaluate the differences in serum cytokine levels between survivor and non-survivors.

Results: Among the 40 patients included, a significant positive correlation was found between multiple cytokines. Serum levels of IL-6, IL-10, and tumor necrosis factor alpha in non-survivors were consistently elevated compared to that of the survivors. Kinetic variations of IL-6, IL-8, and IL-10 were associated with a fatal outcome in severe patients with COVID-19, independent of sex, age, absolute lymphocyte count, direct bilirubin, hypertension, chronic obstructive pulmonary disease, and cancer.

Conclusion: Dynamic changes in serum IL-6, IL-8, and IL-10 levels were associated with survival in ICU and could serve as a predictive biomarker in patients with severe COVID-19 to determine therapeutic options.

Key Points

- Cytokines act as biomarkers for determining severity of COVID-19.
- Constant high serum levels of IL-6, IL-8, and IL-10 correlate with poor clinical outcomes.
- The circulating level in IL-6 is an early predictor of disease progression in patients with severe COVID-19.

1 Introduction

The pandemic caused due to coronavirus disease 2019 (COVID-19), has led to more than 30 million infections and 946,000 deaths worldwide. The pathogenesis and effective treatments for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are currently less known. The survival rate of patients with severe COVID-19 in an intensive care unit (ICU) is extremely lower than that of patients with mild COVID-19 patients. Hyperinflammation and pro-inflammatory cytokines have been considered to be key factors in the clinical course of acute respiratory distress syndrome and multiple organ dysfunction syndrome in severe patients with COVID-19 [1] Studies have demonstrated a correlation between clinical and laboratory characteristics and the outcomes in patients with COVID-19 [2,3]. Clinical trials performed on patients with COVID-19, involving targeting cytokine signals, including interleukin (IL)-6, IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF) antagonists, or small molecules, have reported benefits or failure [4-10]. Studies showed that serum cytokine profile predicted COVID-19 severity [11,12]. However, little is known about the correlation between the dynamics of serum cytokine alterations and the different prognoses of patients with severe COVID-19 during their hospitalization. More evidence is needed to determine whether cytokine profiling serves as an optimal biomarker to study inflammation processes and predict the outcome in severely ill patients with heterogeneity [13]. In this retrospective study, we explored the relationship between the kinetic variations of cytokines and the clinical outcomes in patients with severe COVID-19 admitted to an ICU.

2 Materials And Methods

2.1 Study design and participants

This retrospective cohort study was a single-center study and included 40 adult patients with COVID-19 who were admitted to an ICU in Wuhan Leishenshan Hospital in China, from February 19 to April 6, 2020. Wuhan Leishenshan hospital was an emergency specialty field hospital for patients with COVID-19 and managed by Zhongnan Hospital of Wuhan University and a medical team from Shanghai. The diagnosis of COVID-19 was confirmed from the presence of clinical symptoms and of SARS-CoV-2 nucleic acid, using a real-time reverse transcription polymerase chain reaction assay with nasal and pharyngeal swab specimens. All of the individuals with COVID-19 who were admitted to the ICU according to the Chinese Guidelines for the management of COVID-19 (Trial Version 6) [14] were enrolled for this study. The study was performed in accordance with the Declaration of Helsinki and approved by the research ethics commission of Zhongnan Hospital of Wuhan University, Wuhan, China.

2.2 Data collection

Demographic, clinical, laboratory parameters including cytokine test, and outcome data of the ICU patients were obtained from electronic medical records, and the data were checked by two physicians. The normal concentration ranges for IL-1β, IL-8, IL-10, and tumor necrosis factor alpha (TNFα) were below 5 pg/mL, 62 pg/mL, 9.1 pg/mL, and 8.1 pg/mL, respectively. The normal concentration range of IL-6 is between 0–7 pg/mL and

that of normal IL-2 receptor (IL-2R) ranges from 223 to 710 U/mL. The sera of patients were repeatedly assessed and further classified into four groups (≤3d, 4-7d, 8-13d, and ≥14d) according to the length of stay in the ICU, for further analyzing the dynamic variation of cytokines.

2.3 Statistical analysis

We performed all statistical analyses using SAS version 9.4 (SAS Institute, Inc, Cary, USA). The formal hypothesis testing performed was two sided with a significance level of $P<0.05$. Classification variables were expressed as percentage, and the differences between two groups were tested using chi square test. The quantified variables were expressed as mean ± standard deviation, and the differences were analyzed using t-test for normal distribution, while the data were expressed in median and interquartile range (IQR) for abnormal distribution, and the differences were analyzed using the Kruskal-Wallis H test. We used a multivariable-adjusted generalized linear regression model to evaluate the differences in serum cytokine levels between survivor and non-survivor patients, after adjusting for the covariates that were selected using univariate analysis. Figures were drawn using GraphPad Prism version 8.0 (GraphPad Software, San Diego, California, USA).

3 Results

3.1 Demographic and baseline clinical characteristics of patients with COVID-19

A total of 40 patients with severe COVID-19 admitted in ICU were included in this study. The median age was 71 years (IQR 61–81) and 60% of the sample population comprised men. Fever (55%), cough (55%), anorexia or diarrhea (45%), dyspnea (32.5%), and fatigue (32.5%) were commonly reported symptoms in the patients. Comorbidity of malignancy (7.5%), hypertension (47.5%), diabetes mellitus (25%), and chronic obstructive pulmonary disease (10%) were additionally reported. Twenty-two patients (57.5%) were treated using oxygen therapy including nasal cannula and mask oxygen. Additionally, high-flow oxygen and non-invasive ventilation (22.5%), tracheal intubation (20%), and extracorporeal membrane oxygenation (n = 2) was performed on the patients. The median period from the time of symptom onset to admission into ICU was 20 days (IQR 11–29), and the median time of ICU stay was 8.5 days (IQR 5.25–15.75). The ICU hospitalization of 13 (32.5%) patients was longer than two weeks. From the sample population, 27.5% patients died in the ICU, and 72.5% patients were discharged. The patients were administered antiviral agents (57.5%) and/or antibiotics (92.5%). Nineteen patients (47.5%) underwent corticosteroid therapy; three patients (7.5%) were treated with tocilizumab. Three patients (7.5%) showed a consistently positive test result for SARS-CoV-2 nucleic acid during the ICU stay. Additional details are summarized in Table 1.

3.2 The correlation between time-dependent cytokines and outcomes

We analyzed the relationship between cytokines and explored the effect of longitudinal change of cytokines on the outcomes of severely ill patients in ICU. The concentrations of serum cytokines including IL-1β, IL-2R, IL-6, IL-8, IL-10, and TNFα were measured at different points of time since the patients were admitted to the ICU (Table 2). Among them, IL-1β was observed to be within the normal range in almost all of the patients; this was in agreement with the previous studies. We analyzed the correlation between six types of cytokines and found a significant positive correlation (Table 3). IL-2R had the highest correlation with TNFα (r = 0.69652, $P< 0.0001$) and IL-6 showed a strong correlation with IL-10 (r = 0.63088, $P< 0.0001$). The dynamic changes in cytokine profiles of survivors and non-survivors are illustrated in Figure 1. The serum levels of IL-6, IL-10, IL-8, and TNFα in non-survivors showed a gradual increase compared with that of the survivors during the ICU stay. Upon admission to the ICU, the serum levels of IL-6 was significantly high in non-survivors. The serum levels of IL-10 showed statistically different between survivors and non-survivors after three days committed to ICU. The IL-8 serum levels were statistically different in two weeks of hospitalization ($P< 0.05$).

3.3 IL-6, IL-8, and IL-10 are associated with the survival of patients with severe COVID-19 admitted in ICU

To further explore the role of cytokines in the outcome of severe patients with COVID-19, we used a generalized linear regression model to analyze the differences in serum cytokine levels between two outcome groups (survivor vs. non-survivor). The following factors were considered for analysis: age, gender, absolute neutrophil count, absolute lymphocyte count, direct bilirubin, lactate dehydrogenase, blood urea nitrogen, C-reactive protein, and D-dimer, as well as the history of hypertension, diabetes mellitus, chronic obstructive pulmonary disease, and cancer [13,14]. By using univariate analysis, we found that age ($P< 0.001$), absolute lymphocyte count ($P= 0.005$), direct bilirubin ($P< 0.001$), history of hypertension ($P< 0.001$), chronic obstructive pulmonary disease ($P= 0.002$), and cancer ($P< 0.001$) were associated with the outcome and were included in the next analysis (Table 4 and Online Resource). We further found that IL-6, IL-8, and IL-10 were associated with the outcome in patients with severe COVID-19 after factoring sex, age, lymphocyte count, direct bilirubin, history of chronic obstructive pulmonary disease, cancer, and hypertension into the next analysis (Table 5).

4 Discussion

The prevalence of COVID-19 and the resulting high fatality in the ICU are major problems. Finding an ideal therapeutic strategy for patients in the ICU has been a challenge under the current circumstances. Identifying biomarkers that are produced during the course of the disease would be helpful for guiding the treatment and supervising the response. Cytokine storm syndrome (CSS) or hyperinflammation has been recognized in the pathogenesis of COVID-19. Several studies have shown that different inflammatory cytokines are correlated to the severity of the disease and survival of patients [15,16]. This study provided evidence that the kinetic variation of IL-6, IL-8, and IL-10 was closely associated with the prognosis in patients with severe COVID-19, independent of other risk factors including demographics, comorbidities, and common laboratory markers.

It has been reported that IL-6 is a potent predictive biomarker in patients with COVID-19 [11,17-18]. IL-6, produced by multiple cell types including monocytes and macrophages, is shown to be involved in inflammation and immune response and plays a role in CSS or cytokine release syndrome (CRS). Blocking IL-6 has been a main focus in mitigating cytokine storm-related symptoms in patients with severe COVID-19. Nevertheless, regarding the clinical symptoms of COVID-19, including lung injury and the circulation of various cytokines, the cytokine profiles in patients with COVID-19 were distinct from those of typical cytokine storm including CRS, hemophagocytic lymphohistiocytosis, or macrophage activation syndrome [19,20]. IL-6 antagonists, which have been approved for treating chimeric antigen receptor T cell-induced CRS, have demonstrated various effects on patients with COVID-19 in multiple studies [21]. It is difficult to draw conclusions because of the variable participants and intervention criteria. As the most common adverse event, serious infections were additional concerns. Soners et al. found that tocilizumab is associated with an increased proportion of superinfections in mechanically ventilated patients with COVID-19 [22]. It is of importance that who might benefit from these therapy regimes considering heterogeneity of patients with COVID-19. Rossotti et al. performed a retrospective, single-center analysis on 74 patients treated with tocilizumab and 148 matched controls [23]. They found that a steep increase in IL-6 levels in patients indicated a poor clinical outcome. Similarly, Quartuccio et al. showed that out of 24 patients who received tocilizumab treatment, 6 patients died, and 24–48 h post-tocilizumab treatment, IL-6 serum levels were significantly higher in non-survivors than in survivors [24]. Two studies found that low levels of IL-6 at baseline were associated with a response to sarilumab or tocilizumab treatment [25,26]. Together with our results, supervising the dynamic changes in serum IL-6 levels might offer an opportunity to define inclusion population, predict the prognosis, and adjust the treatment regime in clinical trials or off-label treatment with IL-6 antagonists.

We found that continuous elevated level of serum IL-8 in non-survivors compared to the survivors despite statistic difference was only observed in patients after two weeks' hospitalization. IL-8 is normally secreted by multiple cells exposed to stimuli and is considered to be the primary molecules of acute inflammation. Increased neutrophils have been thought to correlated to the severity of patients with COVID-19 in the previous clinical observation [27]. IL-8 has the effect on potent neutrophil chemotactic and promotion of angiogenesis, might result in deterioration of COVID-19. IL-6 signaling promotes IL-8 recruitment and enhances the inflammation cascade.

We found that the increase in serum levels of IL-10, which is normally considered an anti-inflammatory molecule, was correlated with the increase in levels of pro-inflammatory factors including IL-6 and was also a predictor of survival in the ICU patients regardless of other severity factors. SARS-CoV-2 invasion is a trigger that induces a broad range of immunological events. High levels of circulating pro-inflammatory cytokines can lead to tissue damage and multi-organ failure. As a result, a negative feedback loop might be activated to alleviate the response and promote the transition from immune hyperactivity into the resolution phase. Studies show that anti-inflammatory and pro-inflammatory reactions arise concomitantly in patients with sepsis [28,29]. A shortage of IL-10 is indicated by the sustained elevation of TNF, IL-12, and interferon gamma levels in infected mice [30]. Therefore, the increased levels of circulating IL-10 in patients with severe COVID-19 suggested that an immune regulatory network was activated to limit the magnitude of the immune response and repair the damage. The combination of pro-inflammatory and anti-inflammatory mediators represents the immunological pathogenesis of SARS-CoV-2 infection. Ying et al. reported that IL-6, IL-7, IL-10, IL-18, granulocyte colony stimulating factor (G-CSF), macrophge colony stimulating factor (M-CSF), monocyte chemoattractant protein-1 (MCP-1), MCP-3, interferon gamma-induced protein *10 (IP-10),* monokine induced by gamma (MIG), and macrophage inflammatory protein 1 alpha (MIP1α) were associated with the severity of COVID-19 [31]. In another study that enrolled patients with moderate and severe COVID-19, IL-6 and IL-10 were used as predictors for recognizing patients at high risk of deterioration [12].

Our study is the first to show that the dynamic variation of specific cytokine profiles is correlated with the outcome of patients with COVID-19 admitted in the ICU. Limitations of this single-center study involve the small sample size and missing laboratory parameters that may induce potential bias. We observed the survival of patients during their ICU stay; this might slightly differ from the final clinical outcome. Notwithstanding, our results signified that circulating cytokine biomarkers might prove useful for evaluating therapeutic response and optimizing the therapeutic strategies in patients with severe COVID-19.

Declarations

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Compliance with ethical standards

Disclosures No potential conflict of interest was reported by the authors.

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Tables

Table 1 Demographics and baseline laboratory characteristics of the patients with COVID-19 in the ICU

Characteristics	Reference range	Parameters
Demographic		
Age (years)		71 (61–81)
Gender (male %)		24 (60%)
Days from symptom onset to admission to ICU		20 (11–29)
Days in ICU		8.5 (5.25–15.75)
Comorbidity		
Diabetes		10 (25%)
Malignancy		3 (7.5%)
Hypertension		19 (47.5%)
Chronic obstructive pulmonary disease		4 (10%)
Laboratory characteristics		
Absolute neutrophil count (per mL)	1800–6300	5900 (3600–8700)
Absolute lymphocyte count (per mL)	1100–3200	810 (600–1300)
Total bilirubin (mmol/L)	2–26	10 (6.8–16)
Lactate dehydrogenase (U/L)	125–243	264 (217–387)
Blood urea nitrogen (mmol/L)	2.8–7.6	7.9 (5.2–14)
D-dimer (mg/L)	0–0.55	2.5 (1.4–4.4)
C-reactive protein (mg/L)	0–4	27 (9–54)

Data are expressed as mean ± SD, median (interquartile range), or n/N%.

COVID-19, coronavirus disease 2019; ICU, intensive care unit

Table 2 Serum level of cytokines in patients with severe COVID-19 at different points of time

		D1-3		D4-7		D8-13		D ≥14	
	Reference	Survivor	Non-survivor	survivor	Non-survivor	survivor	Non-survivor	survivor	Non-survivor
Cytokines	Range	(n=29)	(n=11)	(n=29)	(n=11)	(n=29)	(n=11)	(n=29)	(n=11)
IL-1b (pg/mL)	<5	5.00	5.00	5.00	6.00	5.00	5.00	5.00	5.00
		(5.00-5.00)	(5.00-5.00)	(5.00-5.00)	(5.00-8.46)	(5.00-5.00)	(5.00-9.70)	(5.00-5.00)	(5.00-5.00)
IL-2R (U/mL)	223-710	844.50	807.00	681.50	2852.50	1125.50	3469.50	1577.00	2148.50
		(635.00-1054.00)	(661.00-2953.00)	(487.50-960.50)	(1873.00-3467.50)	(474.50-1380.00)	(457.50-4442.50)	(1274.00-1838.00)	(364.00-3933.00)
IL-6 (pg/mL)	0-7	10.36	230.30	29.80	161.90	95.18	4080.50	20.69	664.00
		(7.85-21.86)	(75.50-252.20)	(13.85-68.69)	(101.73-1725.95)	(11.77-161.00)	(2531.00-5000.00)	20.69(7.52-80.33)	(664.00-664.00)
IL-8 (pg/mL)	<62	12.50	29.00	11.00	25.50	17.50	31.00	19.00	56.5
		(7.00-17.00)	(18.50-35.00)	(9.00-19.00)	(20.00-47.50)	(8.00-32.50)	(24.00-64.00)	(10.00-22.00)	(53.00-60.00)
IL-10 (pg/mL)	<9.1	5.00	8.20	5.00	12.90	6.15	21.10	15.20	17.85
		(5.00-5.00)	(5.00-8.90)	(5.00-5.15)	(8.40-28.05)	(5.00-11.90)	(15.90-32.55)	(9.70-19.00)	(16.30-19.40)
TNFa (pg/mL)	<8.1	10.10	9.10	7.65	15.50	10.85	24.15	18.60	13.95
		(7.20-13.70)	(9.10-10.40)	(6.30-9.75)	(13.65-19.55)	(7.65-14.4)	(13.75-36.10)	(10.70-23.10)	(10.60-17.30)

IL-1b, interleukin 1 beta; IL-2R, interleukin-2 receptor; IL-6, interleukin 6; IL-10, interleukin 10; TNFα, tumor necrosis factor alpha

Table 3 Correlation analysis between the circulating levels in cytokines in patients with severe COVID-19

	IL-1b	IL-2R	IL-6	IL-8	IL-10	TNFα
IL-1b	1	0.21655	0.0935	0.30113	0.36974	0.426
P value		0.0965	0.4773	0.0194	0.0036	0.0007
IL-2R	0.21655	1	0.31517	0.46515	0.38548	0.69652
P value	0.0965		0.0142	0.0002	0.0024	<0.0001
IL-6	0.0935	0.31517	1	0.4547	0.63088	0.39359
P value	0.4773	0.0142		0.0003	<0.0001	0.0019
IL-8	0.30113	0.46515	0.4547	1	0.31438	0.59501
P value	0.0194	0.0002	0.0003		0.0144	<0.0001
IL-10	0.36974	0.38548	0.63088	0.31438	1	0.52021
P value	0.0036	0.0024	<0.0001	0.0144		<0.0001
TNFα	0.426	0.69652	0.39359	0.59501	0.52021	1
P value	0.0007	<0.0001	0.0019	<0.0001	<0.0001	

IL-1b, interleukin 1 beta; IL-2R, interleukin-2 receptor; IL-6, interleukin 6; IL-10, interleukin 10; TNFα, tumor necrosis factor alpha

Table 4 Demographics and baseline laboratory characteristics of the patients in ICU with different outcomes

Characteristics	Reference Range	Survivior（n=29）	Non-Survivior（n=11）	*P* value
Clinical characteristics				
Age (years)		62 (72-83)	61(47-73）	<0.001
Gender (male）		19 (65.5%）	5 (45.5%)	0.13
Comoribidity				
diabetes		22 (75.9%)	7/11(63.6 %)	0.39
Malignancy		1/29 (3.4%)	2/11(18.1%)	<0.001
Hypertension		15/29 (51.7%)	4/11(36.4%)	<0.001
Chronic obstructive pulmonary disease		3/29 (10.3%)	1/11(9.1%)	0.002
Laboratory characteristics				
Absolute neutrophil count (per mL)	1800-6300	5940 (3530-7750)	6080 (2370-9340)	0.37
Absolute lymphocyte count (per mL)	1100-3200	820 (670-1130)	250 (220-1050)	0.005
Direct bilirubin (umol/L)	0-7	3.60 (2.60-5.95)	9.95 (5.80-19.15)	<0.001
Blood Urea Nitrogen (mmol/L)	2.8-7.6	7 .00 (4.85-13.15)	9.95 (5.80-19.15)	0.32
Lactate dehydrogenase (U/L)	125-243	259.5 (214.3-348)	347 (235.5-477.5)	0.39
D dimer (mg/L)	0-0.55	2.12（1.17-3.86）	3.06（1.43-7.49）	0.16
C reaction protein (mg/L)	0-4	22.66（5.98-42）	138 (87.9-138.0)	0.16

Table 5 Mean difference and 95% confidence interval in change in six cytokines according to outcome groups

Cytokines	Survivors (n=29)	Nonsurvivors (n=11)	*P* trend
IL-1b	Ref (0)	0.03 (-0.04, 0.09)	0.44
IL-2R	Ref (0)	0.06 (-0.19, 0.31)	0.66
IL-6	Ref (0)	1.27 (0.72, 1.82)	<0.001
IL-8	Ref (0)	0.29 (0.008, 0.58)	0.04
IL-10	Ref (0)	0.22 (0.01, 0.43)	0.04
TNFa	Ref (0)	0.09 (-0.08, 0.27)	0.27

1. Abbreviation: IL-1b, interleukin 1 beta; IL-2R, interleukin-2 receptor; IL-6, interleukin 6; IL-10, interleukin 10; TNFα, tumor necrosis factor alpha
2. All the cytokines were log-transformed.
3. The model was adjusted for sex, age (y), lymphocyte (/mL), direct bilirubin (mmol/L), chronic obstructive pulmonary disease (yes ***vs.*** no), cancer (yes ***vs.*** no), hypertension (yes ***vs.*** no), diabetes mellitus (yes ***vs.*** no).

Figures

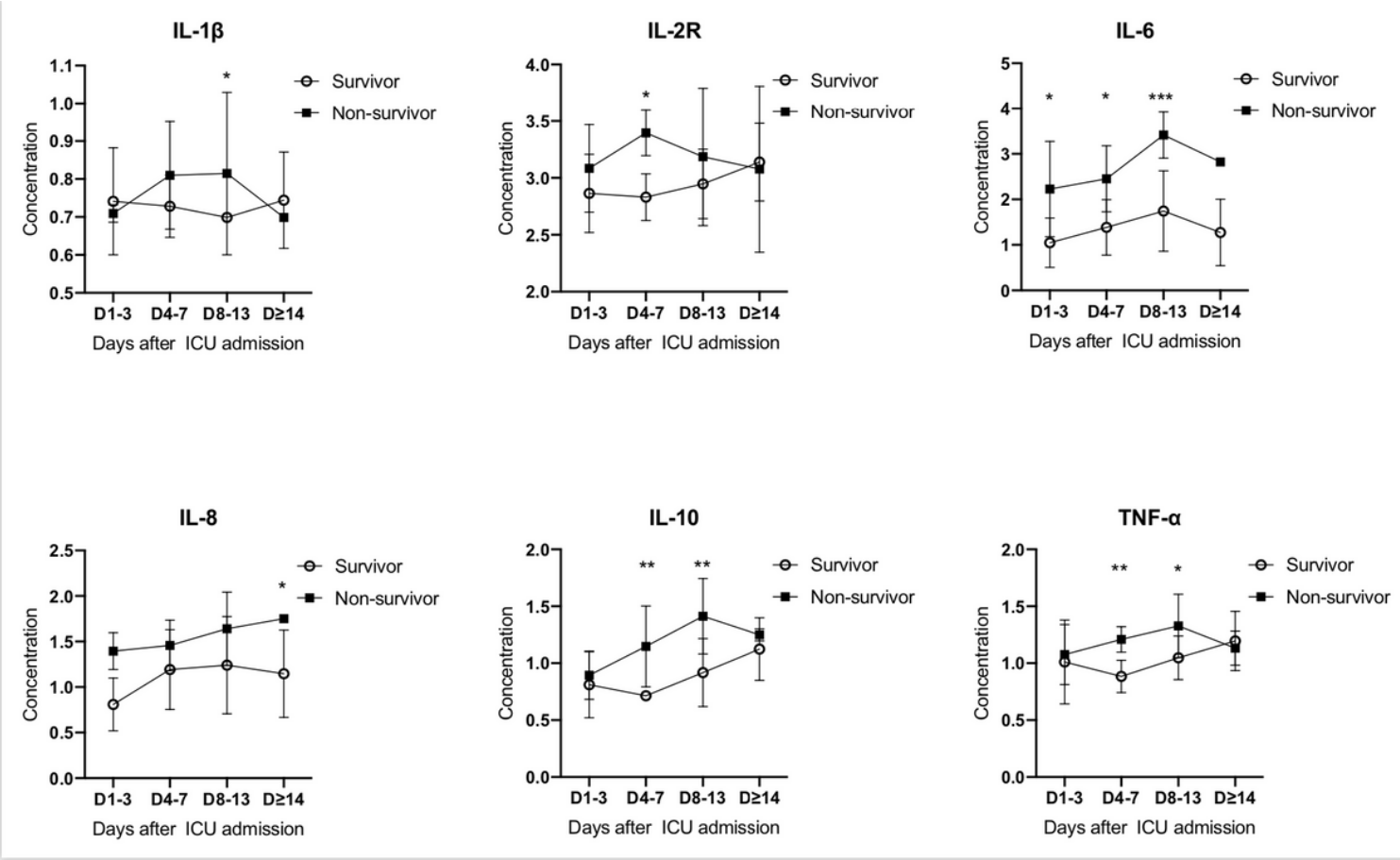

Figure 1

Dynamic changes in cytokine profiles of patients with severe COVID-19. The concentrations of circulating IL-1β, IL-2R, IL-6, IL-8, IL-10, and TNFα were analyzed at different time points after admission in the ICU. Data are presented as mean + SD. The significant difference between survivors and non-survivors was compared using the Kruskal-Wallis H test and indicated as *P < 0.05, **P < 0.01, and ***P < 0.001. This figure was drawn using GraphPad Prism version 8.0. IL-1β, interleukin 1 beta; IL-2R, interleukin-2 receptor; IL-6, interleukin 6; IL-10, interleukin 10; TNFα, tumor necrosis factor alpha

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